Monosodium Glutamates #39

39

1. Ebert, A.G. Toxicol Appl. Pharmacol 17 274 (1970) 2. Lemkey, Johnston, N. et al Abstract Presented Before the 1974 Anatomists Meeting Cleveland, Ohio 3. Evaluating the Safety of Food Chemcials NAS-NRC Washington, D.C. 1970

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : Hearing Clerk, HFC-20

DATE: August 8, 1974

FROM : HFF-335

SUBJECT: Additional material for Scientific Literature Reviews

We attach the following new material for inclusion in the appropriate GRAS substances Scientific Literature Reviews:

1. Material for glutamic acid. Review from Dr. Ebert.
2. Material for Sorbic Acid Review from Monsanto.

Please acknowledge receipt.

Alan T. Spiher, Jr.
Chief, GRAS Review Branch

Attachments
Glutamic acid review
Sorbic acid review

Hearing Clerk.

INTERNATIONAL GLUTAMATE TECHNICAL COMMITTEE

EUROPE - Dr. T. A. Giacometti
JAPAN - Dr. T. Tsunoda
TAIWAN - Dr. T. C. Tung
U.S.A. - Dr. A. G. Ebert

PLEASE REPLY TO:
85 Walnut Street
Watertown, MA 02172

June 11, 1974

Bureau of Foods
Food and Drug Administration
GRAS Review Branch (BF-335)
200 "C" Street S.W.
Washington, D.C. 20204

Attn: Mr. Ronk

Dear Mr. Ronk:

As a follow up to this Committee's filing of data on glutamate on May 30th, we would appreciate your adding the three papers attached, which although mentioned in the bibliography, were not included in the copies of the papers we submitted:

Attached please find three copies of:

1. Ebert, A.G. Toxicol Appl. Pharmacol 17 274 (1970)
2. Lemkey, Johnston, N. et al Abstract Presented Before the 1974 Anatomists Meeting, Cleveland, Ohio
3. Evaluating the Safety of Food Chemicals NAS/NRC Washington, D.C. 1970.

We would appreciate your applying this material to the data submitted May 30th on glutamate.

Sincerely yours,

A. G. Ebert

Chairman

A. G. Ebert, Ph.D/rbj1/4

Attachment

Chronic Toxicity and Teratology Studies of L-Monosodium Glutamate and Related Compounds. A. G. Ebert, Growth Sciences Center, International Minerals and Chemical Corporation. (Sponsor: R. J. Weir.)

Two year feeding studies of L-monosodium glutamate (L-MSG), DL-monosodium glutamate (DL-MSG) and L-glutamic acid (L-GA) were carried out using Sprague-Dawley rats (75 animals of either sex/group + 150 controls) and C-57 black mice (100/group, all males + 200 controls). Rats received diets containing 0.1% or 0.4% of test compound; mice 1% or 4%. Possible adverse effects on reproduction and the teratogenic potential of L-MSG were measured in rabbits (New Zealand White variety) following 2-3 weeks of feeding at 0.1%, 0.825% and 8.25% of diet. There were 22-24 does/group and 16 males/group, all having sired or produced one litter prior to test compound feeding. Test compound feeding was continued in pregnant rabbits throughout gestation.

In rats, there were no differences between treated and control groups with respect to weight gains, food intake, hematology, gross and histopathology at any of the 3 intervals studied (241, 567 and 681 days). Survival rates at 2 years averaged 59% over all groups (range 53-63%). Gross abnormalities, seen with increasing age, included a white film over the cornea and a high incidence of fibrous tumors. Presumed tumor incidence at completion of the 730 day test averaged 40.1% across all treatments, 42.4% in controls. The most common microscopic lesion, seen in all groups, was a low grade inflammatory response in lungs,

kidneys and spleen.

In mice, however, only a single benign adenoma was found in controls or all L-MSG, DL-MSG, and L-GA treated animals. Gross and histopathology suggested renal effects in DL-MSG treated animals. Other findings were within normal limits with the exception of low two year survival rates (20% overall) due to the combatant nature of this strain of mouse.

L-MSG, at all levels studied, was without deleterious effects on reproduction of rabbits with respect to number of fetal resorptions, birth rate, survival rate at weaning, presence of internal and external abnormalities and skeletal development.

Mr. Chairman:

In the year since the last meeting of this Society, considerable attention has been drawn, largely in the lay press, to a diverse group of compounds well known to many toxicologists. I am referring to food additives and, in particular those items classified as Generally Recognized As Safe, that is on the GRAS list. Among the remarks made against the efficacy and safety of a number of these compounds were the claims that L-monosodium glutamate or MSG caused the transient neuromuscular episode in humans commonly referred to as the Chinese Restaurant Syndrome and, that the compound induced lesions in the developing central nervous system of a variety of laboratory animal species. Further, it was incorrectly stated at Congressional Hearings, that MSG had been accepted because of its natural character and long history of use rather than being toxicologically evaluated, and that such research had, in fact, not been carried out. In reply to numerous inquiries from a variety of concerned individuals we have briefly described in a number of trade and popular publications several of these supposedly non-existent studies. Chronic toxicity studies on MSG sponsored by our corporation were completed in 1952 but had, in fact, not been reported in the archival literature. We thought, therefore, it might be of interest to attendees of this meeting to briefly review results of three of these

older and previously unpublished studies on MSG, if for no other reason than to serve as a background to current studies on MSG the results of which will be reported at this meeting and elsewhere.

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Three studies will be described. Two, 2-year feeding studies were carried out by A. D. Little & Co. in 1950 - 1952. Three compounds were evaluated: L-monosodium glutamate monohydrate, which is the material used as a food flavor enhancer: the monohydrate of the racemic mixture: and the L-form of the corresponding acid.

For each compound studied there were 75 rats per level +150 controls. Feeding began at 3 months of age, markedly older than the weanling age now commonly used as the starting point to begin lifetime feeding studies. Similarly, 100 male mice/level/ compound were studied beginning at 6 weeks of age.

In rats, dietary intake was at the level of 0.1% and 0.4% of total feed, equivalent to 50 or 200 mg/Kg/day, as the former represents the most common use level of MSG. The latter covers the higher use levels.

Mice received diets at 10X the concentration used in the rat studies, namely, 1% and 4% of diet, equivalent to a dose of 1.65 or 6.60 g/Kg/day. This study was designed

in part to give data at levels far greater than those used in food and also because MSG was of interest at the time as a therapeutic agent that might improve the IQ of mentally retarded children. Dietary levels of 1% to 4% roughly approximated the therapeutic regimen under evaluation which included doses of up to 45 grams per child per day.

At Hazelton laboratories in 1966, a teratology study was carried out in rabbits after feeding L-MSG at levels of 0.1% to 8.25%. This was the equivalent of up to 2.5 g/Kg/day. Males and females were fed 3 weeks prior to mating and females throughout gestation. Negative and positive controls (Thalidomide 100 mg/Kg/day) given from day 8 to 16 of gestation, were also studied. There were 40 animals per group.

Hematologic examinations were made on randomly selected rats and mice from each test group in the first two studies. The typical hematologic determinations of erythrocyte and total and differential leukocyte counts, plus hemoglobin were carried out. All values were within normal limits with the possible exception of a slight increase in monocytes especially in older animals. This finding was ascribed to low grade infections in older animals and will be seen again later.

Clinical Chemistries and organ function tests, often

characteristic of contemporary chronic toxicity studies are absent from these studies primarily because these techniques were yet to be developed when this research was conducted. As to results:

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Body weight determinations were made at 10 intervals throughout the 2 year course of the rat feeding study. No differences were observed in the period following approximately 200 days of test compound feeding, however each of the test compounds, $_{L}$-MSG, $_{DL}$-MSG & $_{L}$-GA, induced weight increases at the time intervals earlier than 200 days. As can be seen from the top figures, the growth response occured primarily in male rats. A separate short feeding study was therefore carried out in weanling rats to determine if growth acceleration was associated with increased food consumption. Feeding of each of the test compounds at the 0.4% level resulted in increased consumption of food throughout the 42 day course of the food consumption study.

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At 6 months and one year, survival rates ranged from 93 to 98%. There was no effect on survival rates that could be attributed to any of the test compounds at any of the time intervals. At the conclusion of the study survival rates ranged from 53 to 63%.

Animals that expired during the course of the study were subjected to gross and histopathologic examination except where prevented by autolysis or cannibalism. At several intervals, asymptomatic representatives from each test group were sacrificed for gross and histopathologic study, as were all survivors at the end of the 2 year study.

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Any abnormal appearing organ or tissue was preserved for microscopic evaluation. Organs and tissues routinely studied included: trachea, thyroid, esophagus, stomach, intestine, heart, lungs, liver, kidney, spleen and gonads. Conspicuous by their absence, according to modern protocols, were brain, thymus from young animals, muscle and fat samples. Organs and tissues grouped under "other" included reproductive tracts, adrenals and urinary bladder.

There was no predisposition to toxic manifestations in a given organ in any of the groups. As illustrated, gross and histopathologic findings of visceral organs were largely associated with the lungs, kidneys and spleen. On histopathologic examination, lungs showed alveolar fibrosis, lymphocytic accumulation around the bronchi, and other evidence of low grade infections in both control and treated rats. Kidneys were also characterized by evidence of low grade, chronic infection including dilated renal tubules, localized nephritis and foci of congestion in the cortex.

Recall that evidence of low grade infection was reported in the hematologic findings. Spleen involvement, included congestion and disruption of the cellular architecture, characteristic of old animals. Necrosis and general erosion of gut epithelia was observed. A low incidence of abscesses and isolated zones of hepatic necrosis was also observed. Two cases of cirrhosis were reported in the 0.1% $_{DL}$-MSG group.

Throughout the second year of the study a high incidence of localized swellings referred to as "tumors" was observed throughout all groups. The actual and percentage incidence of these swellings in each test group are illustrated in the last column. Although subsequent gross and microscopic examination indicated that a number of the swellings were cyst-like, containing a milky exudate, (that is, not verified as tumors), all swellings were referred to as presumed tumors. The highest incidence was observed in the 0.1% $_{L}$-MSG rats, however a dose response effect was not observed as the 3rd lowest incidence was in the 0.4% $_{L}$-MSG rats. The percentage incidence of swellings across all test rats averaged 40.1%, slightly lower than the 42.4% observed in controls.

There was a marked sex-linked difference in the locations and type of swellings. The incidence in female rats was

4 times that of males. In females the incidence of swellings on the ventral surface was about 10X that observed on the dorsal. However in males they were essentially evenly distributed. In females the growths were associated with mammary gland proliferation. No growths were observed within the visceral cavity of the rats.

Drs. Grey and Fogg, the toxicologist and pathologist involved in this study microscopically examined 90 of these growths. They reported that all but 3 of the tumors were benign. Forty-nine of the tumors were adenomas, 11 were fibrosarcomas, 10 were mixed and 6 were fibraodenomas. As with the incidence of total tumors, no type of tumor predominated in a given treatment group. There were 3 carcinomas, 1 in the 0.1% racemic mixture group, 2 in controls.

Although this effect could be explained on the basis of a natural phenomenon in the strain of rat used, it was deemed advisable to repeat the study using a species of animal resistant to the formation of spontaneous tumors but sensitive to known chemical carcinogens.

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The C-57 black mouse was therefore chosen and the dietary concentrations increased to 1% and 4% of each test compound. As in the studies with rats, survival rates and

values for hematologic observations were similar between treated and control mice. Body weight data were not obtained. Organs routinely examined were trachea, thyroid, submaxillary glands, lungs, heart, stomach, liver, intestine, spleen, pancreas, kidneys and skin. As in the rat studies, brain, thymus and muscle samples were not obtained. The incidence of gross pathologic findings is illustrated. Histopathologic findings in visceral organs were similar to the findings in rats. Evidence of low grade infection and tissue degeneration characteristic of the old animal was again seen across all groups.

Of greatest interest, in light of the results in rats, was the incidence of swellings at the end of the 2 year feeding. Six of the eight swellings found were, in fact, lumps under the skin in the region of the genitals and were external to the body wall, The report suggested that the swellings were traumatic, induced by fighting in the colony, because the mice were caged in groups. Microscopic examination indicated the swellings were cysts filled with viscid-yellow material. A single case of thyroid enlargement was reported.

The single tumor to be reported was not detected on gross examination but was discovered in a lung prepared for routine histopathologic study. The growth was defined as a benign adenoma and it occurred in the 1% $_{DL}$-MSG group.

As to the teratology study, breeding data are illustrated

on the next slide. Recall that $_{L}$-MSG was studied at three levels the equivalent of from 30 mg/Kg to 2.5 g/Kg/day. Means ± standard errors plus totals for each of the parameters are illustrated. There were no differences between the various $_{L}$-MSG treated groups in numbers of implantation sites, number of resorptions, litter sizes, incidence of still-births, or birth weights. Somewhat surprisingly, Thalidomide at 100 mg/Kg/day also was without effect.

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Not less that 48 pups/treatment were cleared and stained with KOH, glycerin and alizarin for study of any skeletal deformities. Both live and stillborn pups were examined. The highest incidence of effects on skeletal development included missing or small 5th sternbrae and the presence of additional rib formation seen throughout all groups. The report states that these findings occured at approximately the same incidence reported as the norm for this animal. In one litter of rabbits on 8.25% MSG, 3 animals showed a retardation of closing of the cranial suture. As no other litter at this dosage showed any sign of this effect it was considered to be not compound induced. Thalidomide was again without effect, possibly due to an insufficient dose.

In Summary.

$_{L}$-MSG monohydrate, $_{DL}$-MSG monohydrate and $_{L}$-glutamic acid were evaluated in 2 year feeding studies in rats and mice. In rats, each of the test compounds produced a slight acceleration of growth over negative controls early in the study, but not after 200 days. Findings on mortality rates, hematology, gross and histopathology were within normal limits. A high incidence of swellings was observed in treated and control rats during the second year of the study. A number of these growths proved to be benign adenomas suggesting the need for further research. Such research was completed in a carcinogen sensitive strain of mouse utilizing 10X the dietary concentration that had been utilized in the research on rats. Only a single neoplasia was observed. In a third study $_{L}$-MSG at dietary levels of up to 8.25% was without teratogenic effect in rabbits.

I would comment in conclusion on one other aspect of these studies. Following the report in Science suggesting that subcutaneously administered MSG at doses of 0.5 to 4 g/Kg to 2 - 9 day old mice induced lesions in the central nervous system and the subsequent interpretation by the lay press that MSG constituted a "Peril in Pregnancy" we asked Dr. Voelker of Hazelton to examine the brains of the formalin preserved pups that had been delivered by Caesarian

section in the teratology study just described. This work was completed using 10 pups from each dose level of MSG. Brains were paraffin imbedded and stained with H & E. Transverse sections were examined at the level of the hypothalamic nuclei and pituitary. It is to be emphasized that the protocols differed. In rabbits, MSG had been orally administered to the mothers so that pups received glutamate via placental circulation, whereas in mice MSG was subcutaneously administered to the newborn pups. Nevertheless, Dr. Voelker was convinced that the method he employed was adequate to reveal any lesions in that area of the rabbit brain reportedly effected in studies with mice. Evidence of neuronal necrosis or other pathologic alterations was not apparent in any of the MSG treated or control animals. The only pathology finding was hydrocephaly in pups from thalidomide treated does.

I am indebted to Dr. Kensler of A. D. Little, Drs. Holsing and Voelker of Hazelton and Dr. Weir now at Bionetics for their assistance in providing additional background information to the formal reports just described.

BREEDING DATA

Rabbits

TEST COMPOUND	# PREGNANT	# IMPLANTATION SITES	# RESORPTIONS	# ALIVE	# STILLBIRTHS	MEAN LITTER WT. (g)
Neg.Control	21/24	8.0±3.2 168	0.9±0.1 19	6.3±3.2 132	0.8±0.9 17	44.3±9.4
Thalidomide 100 mg/Kg/Day (Day 18-24)	18/22	8.2±2.3 148(6)*	0.5±0.7 8	6.8±2.3 109	1.0±1.2 25	41.3±6.2
L-MSG 0.1%	19/24	9.5±1.6 181	0.6±1.0 12	8.4±1.9 160	0.5±0.9 9	38.2±6.5
L-MSG 0.825%	20/24	8.7±2.0 173	0.7±1.0 14	7.6±2.1 151	0.4±0.7 8	39.6±8.9
L-MSG 8.25%	18/24	9.3±1.6 168	0.4±0.7 8	8.0±1.6 144	0.9±1.2 16	40.5±5.5

(*No. unaccounted for)

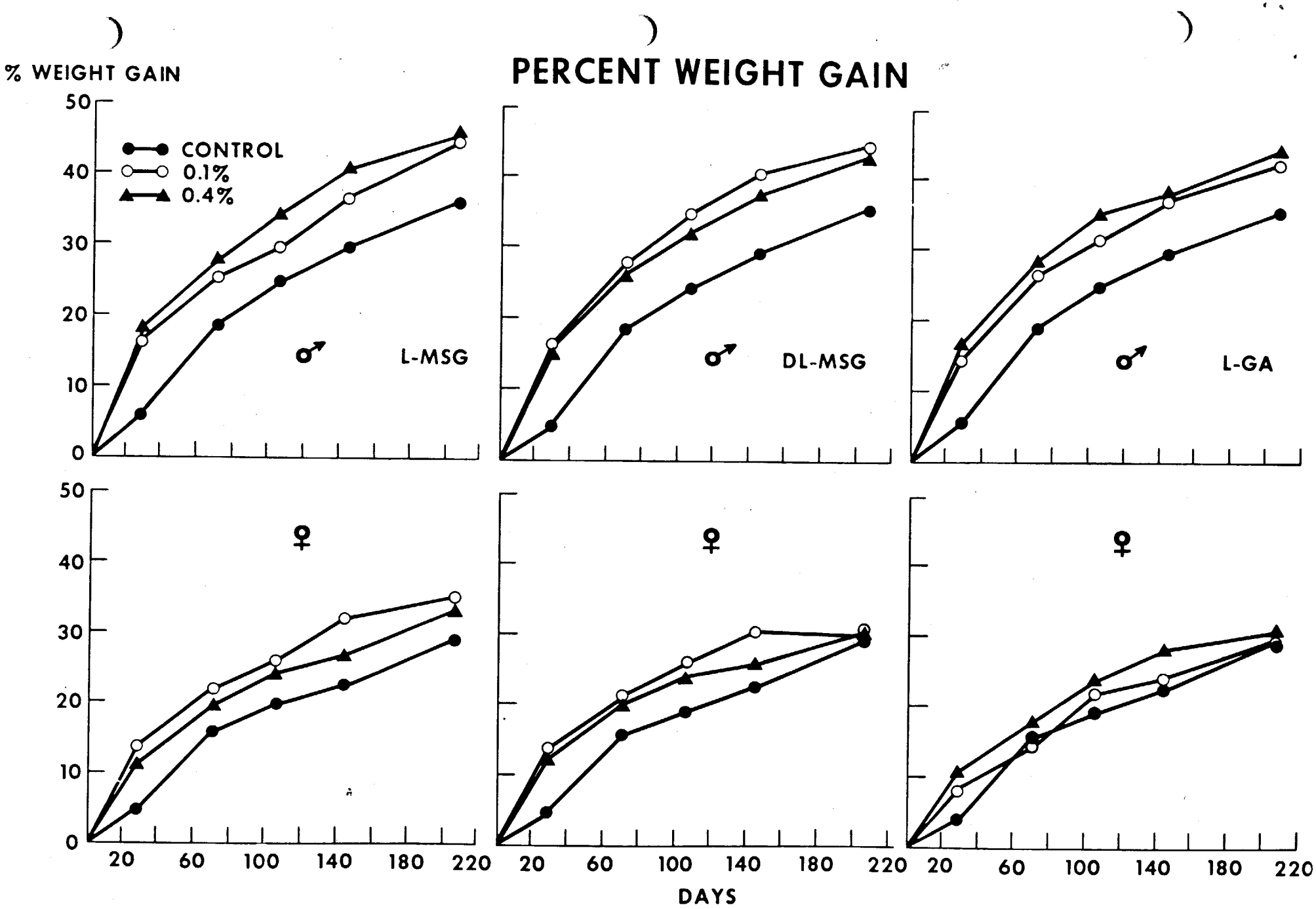
SUMMARY OF STUDIES

COMPOUND	ANIMALS	LEVELS IN DIET (%)	TEST	LABORATORY
L-monosodium glutamate·H_2O DL-monosodium glutamate·H_2O L-glutamic acid	Rats, Sprague-Dawley n = 75/level ♂ & ♀ + 50 controls begin 3 months	0.1 (50 mg/Kg/Day)[1] 0.4 (200 mg/Kg/Day)	2 yr feeding	A.D. Little, Inc. (1950-1951)
	Mice, C-57 Black (Jackson) n = 100/level ♂ + 100 controls begin 6 weeks	1 (1.65 g/Kg/Day)[2] 4 (6.60 g/Kg/Day)	2 yr feeding	A.D. Little, Inc. (1951-1952)
L-monosodium glutamate	Rabbits, New Zealand White n = 40 + 40 controls ♂ + ♀	0.1 (30 mg/Kg/Day)[3] 0.825 (250 mg/Kg/Day) 8.25 (2.5 g/Kg/Day)	Teratology (6-7 wk feeding)	Hazleton Labs.Inc. (1966)

[1] Assuming 200 g rat eating 10 g food/day.

[2] Assuming 30 g mouse eating 5 g/day.

[3] Assuming 2 Kg rabbit eating 60 g/day.

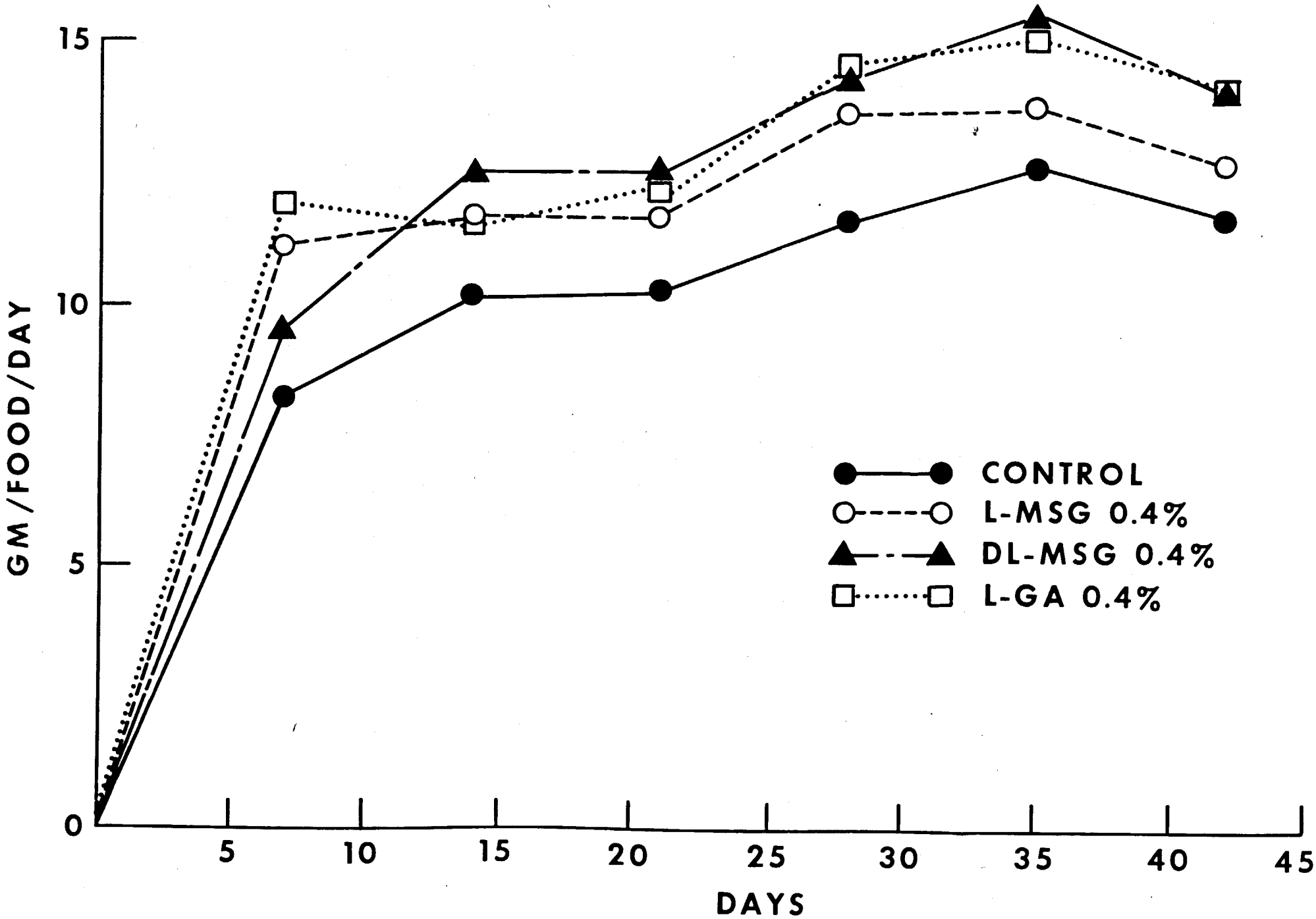

% WEIGHT GAIN
PERCENT WEIGHT GAIN
CONTROL
0.1%
0.4%
L-MSG
DL-MSG
L-GA
♂
♀
0
10
20
30
40
50
20
60
100
140
180
220
DAYS

DISTRIBUTION OF GROSS AND MICROSCOPIC ABNORMALITIES AT TWO YEARS

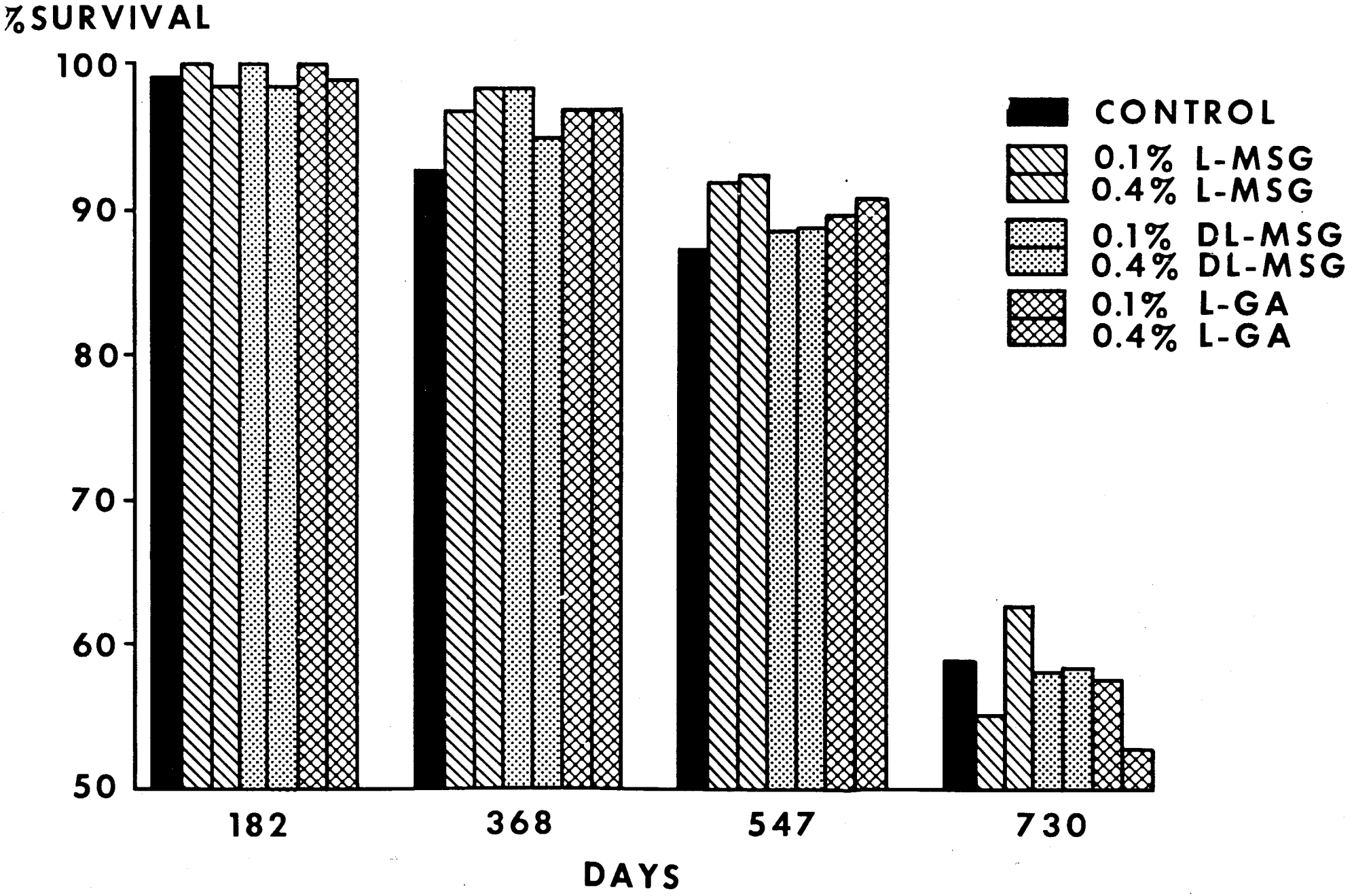
Mice

TEST GROUP	# ANIMALS	# SECTIONS	LUNGS	KIDNEYS	SPLEEN	GUT	LIVER	TESTES	OTHER	"TUMORS"
L-MSG 1%	25	76	9	5	8	3	4	1	3	0
L-MSG 4%	23	84	4	5	5	0	3	4	1	3
DL-MSG 1%	23	105	5	6	4	0	4	3	0	0
DL-MSG 4%	27	103	5	7	5	0	4	4	0	2
L-GA 1%	20	105	6	4	9	2	5	3	3	0
L-GA 4%	29	118	7	1	4	0	2	1	1	0
Control	55	207	11	7	8	0	9	6	4	3

FEED INTAKE - RATS



SURVIVAL DATA



DISTRIBUTION OF GROSS AND MICROSCOPIC ABNORMALITIES AT TWO YEARS

Rats

TEST GROUP	# ANIMALS	# SECTIONS	LUNGS	KIDNEYS	SPLEEN	GUT	LIVER	OTHER	"TUMORS"
L-MSG 0.1%	63	154	18	13	12	3	4	1	41 (54.7)
L-MSG 0.4%	75	162	9	11	8	6	2	3	30 (39.6)
DL-MSG 0.1%	65	190	7	7	7	1	3	3	22 (29.0)
DL-MSG 0.4%	69	173	10	13	9	1	4	2	26 (34.4)
L-GA 0.1%	73	236	10	7	8	8	0	2	31 (40.3)
L-GA 0.4%	73	163	14	9	12	6	3	1	32 (42.6)
Control	102	305	14	10	5	4	1	1	64 (42.4)

SKELETAL STAINING

Rabbits

TEST COMPOUND	# CLEARED & STAINED ALIVE	DEAD	SKELETAL ABNORMALITY	NUMBER
Neg.Control	46	9	Rib fusion Spinal curvature Missing 5th Sternbrae Small 5th Sternbrae Additional (13th) rib	1 2 6 27 27
Thalidomide 100 mg/Kg/Day (Day 18-24)	36	6	Missing 5th Sternbrae Small 5th Sternbrae Additional (13th) rib	4 22 6
L-MSG 0.1%	49	5	Rib fusion Missing 5th Sternbrae Small 5th Sternbrae Additional (13th) rib	1 4 44 25
L-MSG 0.825%	45	3	Missing 5th Sternbrae Small 5th Sternbrae Additional (13th) rib	10 26 15
L-MSG 8.25%	46	3	Missing 5th Sternbrae Small 5th Sternbrae Additional (13th) rib Retarded closure of cranial suture	11 34 27 3

LEMKEY-JOHNSTON, N., V. Butler* and W. A. Reynolds, Illinois State Pediatric Institute and Department of Anatomy, University of Illinois, Chicago, Illinois. Brain damage in neonatal mice following high dosages of monosodium glutamate (MSG), N_aCl and sucrose.

Oral administration of MSG at high levels (1-4mg/g) to neonatal mice results in damage to numerous brain areas, including the arcuate-preoptic area, inferior colliculus, hippocampus, anterolateral thalamus, habenula, subfornical organ and area, postrema. (Lemkey-Johnston and Reynolds, 1973).

To ascertain the effects of osmolality in eliciting this damage, we have given oral loads of N_aCl and sucrose solutions at levels equimolar to 4 mg/g of MSG to neonatal mice. Serial sections of entire brains embedded in paraffin were examined. The young mouse (5 days) given N_aCl exhibited edema and pyknotic nuclei in the habenula, caudate-putamen, hippocampus, parahippocampus and cerebral cortex, but not in the arcuate area. The lesions radiated from foci within neural structures in contrast to a band of damage appearing to diffuse inward from cerebrospinal fluid seen characteristically in brains following MSG administration. Mean plasma levels of sodium rose from control values of 130 mEg/l to 151 and 155 mEg/l for MSG and saline-treated neonates, respectively. No lesions were seen in animals older than 6 days or in those mice given sucrose.

The pattern of damage within brain structures differed depending on whether N_aCl or MSG was used, but the type of damage was the same, viz. pyknosis and edema. Thus, the role of high sodium levels plus the extent of immaturity of the neonatal mouse present additional variables to be considered in determining the etiology of the brain lesions following MSG administration.

Evaluating the Safety of Food Chemicals

FOOD PROTECTION COMMITTEE
FOOD AND NUTRITION BOARD
DIVISION OF BIOLOGY AND AGRICULTURE
NATIONAL RESEARCH COUNCIL

NATIONAL ACADEMY OF SCIENCES
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Preface

The Food Protection Committee of the Food and Nutrition Board has, during the past 20 years, periodically reviewed general principles and procedures for evaluating the safety of food chemicals. During that period proposals for improving the assurance of safety have been made by many individuals and organizations. This report is an evaluation by the Subcommittee on Toxicology of the more recently proposed or adopted procedures, against a background of experience with what have become conventional procedures.

Contents

Introduction

The Food Protection Committee, The Joint FAO/WHO Expert Committee on Food Additives, and the U.S. Food and Drug Administration (FDA) have published complementary statements of the principles underlying evaluation of the safety of food chemicals and have set forth proposed procedures for accomplishing the evaluation.[1-10] Recently the FDA has been actively developing protocols as guides for the evaluation of safety of food, drug, and cosmetic chemicals. It is not the purpose of this report to present another set of principles and procedures, but rather to review the purposes and value of what have become conventional procedures and to evaluate in light of that review the contribution or potential contribution that more recently adopted and proposed procedures might make to ensuring the safety of the food supply.

DEFINITION OF TERMS

Natural components of foods are obviously chemical substances. However, the term *food chemicals,** as here used, relates to substances

*The term *food chemical* is used rather than *food additive* because the latter term has specific legal meanings in the United States. Food additive, according to the Food, Drug, and Cosmetic Act, may not include pesticide residues, substances "generally recognized as safe," and color additives.

added to food either directly and intentionally for a functional purpose, or indirectly during some phase of production, processing, storage, or packaging without intending that it remain in, or serve a purpose in, the final product. It does not include either the basic foodstuff itself or chance contaminants. A food chemical may be of natural or synthetic origin; it may be nutritive or nonnutritive and physiologically active or inert. The procedures for evaluating the safety of a chemical for use in food are independent of the reasons for its presence in food.

Toxicity is the capacity of the substance to produce injury. The term includes capacity to induce teratogenic, mutagenic, and carcinogenic effects.

Safety is the practical certainty that injury will not result from the substance when used in the quantity and in the manner proposed for its use.

Hazard is the probability that injury will result from the use of a substance in a proposed quantity and manner.

The evaulation of safety or hazard must take into account the conditions of use of the substance.

BASES FOR EVALUATION OF SAFETY

Safety of a food chemical should be appraised relative to the extent of its proposed use in foods, to the amount that would be consumed under all likely dietary patterns of the populations concerned, to the nature of the biologic response it may induce, and to the minimal intake that might provoke response. No use of a chemical in food should be permitted if there is reason to anticipate that the intake of the chemical from the permitted use could be so high as to produce adverse effects.

Risk–Benefit Relation The decision to use a chemical in such a way that it may remain in the food as consumed should be based on the assurance that the use will be safe (i.e., that there is virtual certainty that no injury will result), and that, directly or indirectly, the use will in some way benefit consumers. Factors to be considered include the following:

The estimated hazard to the consumer
Consumer needs and wishes
The requirements of food supply and public health

The needs of the food producer and processor
The economy, and
The availability of methods and mechanisms for regulatory control

No single formula can relate all these factors to one another under all conditions, but they should be taken into account whenever a decision is being made concerning whether to permit or deny use of a chemical. The risk–benefit relation is affected by the circumstances that pertain to a given situation. For example, if the supplies of a major food staple in a food-deficient area are threatened by pests, use of a chemical to control the pests might well be justified, even though levels of residues greater than those usually permitted remain in the food.

Chemical and Physical Properties It should be possible to characterize the substance in question in chemical and physical terms. Constancy of composition, stability, and freedom from harmful amounts of impurities should be assured. The *Food Chemicals Codex*[11] is designed to provide national standards of identity, purity, and composition for food chemicals, as has been done for drugs in the United States and elsewhere. Standards for food-grade chemicals are described in the *Codex* by chemical and physical specifications that can be attained through good manufacturing practice but that, at the same time, provide reasonable standards of identity and assurance of safety. Obviously, such a compendium is never complete, nor are its listed specifications necessarily permanent; it must be continuously reviewed and revised. Patterns of use of foods and new toxicologic knowledge may be expected to determine the chemicals included as well as their specifications.

The persistence of the chemical in the foods in which it is to be used, its reactivity with the components of these foods, and the identity of the substances to which it may be converted in foods should be known.

Methods for estimation of chemicals in foods must be sufficiently accurate and sensitive to permit estimation of quantities hazardous to health. When conversion products are likely to be hazardous, appropriately accurate and sensitive assay methods for their detection should be available. When, however, the proposed use of a chemical cannot reasonably be expected to result in hazardous amounts being present in food, it is not necessary that analytical methods practicable for routine application to foods in commerce be developed.

Biological Aspects Results of critically designed animal studies of the physiologic, pharmacologic, and biochemical behavior of a proposed food chemical provide the crucial information in the evaluation of safety at a specified level of intake by man. Because of species variation, however, judgment must be exercised in basing a safe level of use by man on data derived from studies with various species of animals. No method for establishing the safety of a food chemical with absolute certainty under all conditions of use is at hand, and none is in sight. Experience has shown, however, that properly conducted and interpreted animal experiments can provide that degree of practical assurance of safety reasonably expected in the evaluation of chemicals for use in human food. In rare instances, adverse responses in animals, discovered well after the material in question had come into regular use, have led to discontinuance of that use, even though no hazard to man had been demonstrated. Nitrogen trichloride, certain synthetic colors, and a few naturally occurring flavoring components, e.g., safrole and coumarin, are examples.

The appropriate study of responses in human beings is a valuable phase of the safety evaluation of chemicals used, proposed for use, or present in food. Occupationally or accidentally exposed subjects provide some data; another possibility is the use of volunteers in controlled studies. The latter should be carried out only after careful investigation of biochemical and toxicologic properties in animals has clearly indicated that the risk to which the subjects may be exposed will be trivial in relation to the benefit of the knowledge to be expected. Data from human studies are especially appropriate in assessing the safety of a material of exceptionally widespread potential use.

Once a food chemical is approved for use, there should be continual observation of the exposed population for deleterious effects that might emerge under prolonged and varying conditions of use, and safety of the use should be reappraised whenever warranted by experience in use or advances in knowledge.

It has been customary to permit use of a new chemical on the basis of long-term feeding studies in at least two species of animals. Chronic toxicologic studies in animals are designed to find a dosage level that will produce a deleterious effect in the animal as a whole or in one or more of its organs. It is not always possible, however, to find a deleterious effect unequivocally related to toxic properties of the substance being studied. In addition to a toxic level, a "no-adverse-effect" level

is sought. This level, adjusted by incorporating an adequate margin of safety, provides an estimated safe level for use in the human diet.

Patterns of Consumption Certain substances that have been in use for many years, either as food chemicals or as natural components of foods, are assessed as being "generally recognized as safe" (GRAS) by experts qualified by scientific training and experience to make that evaluation. Such assessments, like those for any food chemical, should take into account specified or reasonably inferrable patterns of food consumption, for which it may be desirable to consider such regional or national surveys of food consumption patterns as those reported by the U.S. Department of Agriculture.[12] A bulletin on "High Consumption of Foods," prepared by the Agricultural Research Service of the U.S. Department of Agriculture,[13] has stated that "because of increasing need for estimating chemical residues and food additives in foods, there has been growing interest and information on quantities of foods used by *high* consumers, as well as by *average* consumers." High levels of food consumption in this instance are based on data for the ninth decile of the population, i.e., the group representing the highest 10 percent of consumers as distinguished from the remaining 90 percent. These estimated levels are 1.5 to 3.5 times the mean consumption for each broad category of foods.

Adverse reactions that may result from the gross misuse of an otherwise beneficial substance in a food should not militate against its proper use.

Regulatory Aspects The regulatory control of chemicals in foods is normally based in part on the principle that there is a "safe" level of intake for any chemical. In some instances, the high toxicity of a given substance or threat of serious hazard (e.g., carcinogenesis) has led to the setting of "zero" tolerances. The concept "zero" is dependent upon developments in analytical methodology, and a zero tolerance may have little significance from the viewpoint of safety evaluation. In other cases, small quantities of chemicals are known to be present in foods but are treated administratively as "negligible residues," i.e., insignificant or inconsequential insofar as public health is concerned.

It has been proposed that a "zone of toxicologic insignificance" be recognized for administrative purposes. When the maximum level of a chemical likely to be present in food is either calculated on the basis of its proposed technologic use or is analytically determined to be an

amount sufficiently below the safe level, the possibility that injury will result approaches zero. Such a level, which can be established only by qualified experts, is within the zone of toxicologic insignificance, and therefore does not require regulatory action (see Appendix, Guidelines for Estimating Toxicologically Insignificant Levels of Chemicals in Food).

Food additives, as legally defined in the United States, embrace the entire range of substances, the intended use of which may result in their becoming components of or otherwise affecting the characteristics of any food. It is immaterial whether the effect is intentional or unintentional, direct or indirect. Taking into account the broad scope of this definition, Congress provided for certain exceptions, the principal one being to exclude those substances that scientifically qualified experts generally recognize as safe under the conditions of intended use (GRAS) on the basis of either scientific procedures or experience based on common use in food. The effect of this exemption is to use scientific judgment informally in deciding whether it is necessary to require submission of a formal petition, along with supporting evidence of safety, for a regulation to permit and limit the use of a food chemical.

In addition to such common food ingredients as salt, pepper, sugar, vinegar, and baking powder, hundreds of substances have been regarded as GRAS when used in accordance with "good manufacturing practice." The latter term implies that the substances in question are of suitable food grade and that they are used and remain in food in amounts no higher than necessary to accomplish their intended effects. Judgment that a substance is GRAS also takes into consideration the history of the occurrence or use of the substance in food, experience through its use in medicine or in industry, and biochemical, metabolic, and toxicologic evidence relating to the substance itself or to chemically or pharmacologically comparable substances.

Of paramount importance in any evaluations of safety are the conditions of intended use. In addition to the level or concentration of the food chemical, these conditions include the specific food or foods to which it will be added, the amounts and frequency of consumption, and the age, sex, and physiological state of the consuming population.

When all available information is taken into consideration, a GRAS appraisal can provide assurance of safety sufficient to obviate the need for subjecting every food chemical, irrespective of its origin, to the extensive animal testing required to support a food additive petition. Of course, as new knowledge develops relevant to safety evaluation,

as patterns of food consumption change, or as technological uses of a substance in food production and processing change, its status as GRAS should be subject to review.

CURRENT PRACTICE IN AND SCOPE OF TOXICOLOGIC EVALUATION

Current practice in the toxicologic evaluation of food chemicals has evolved from experience with laboratory animals, particularly from knowledge of their nutritional requirements and biological reactions. During the early part of this century studies of the physiology, anatomy, and behavior of the albino rat led to its being selected as the species of choice for nutritional investigations. Research resulting in the discovery of vitamins, which proceeded intensively during the second, third, and fourth decades, led to the development of diets that made it possible to conduct lifetime feeding studies in the rat and, subsequently, in other species.

Toxicologic procedures have evolved further in both design and scope. It is now generally considered desirable to employ at least one nonrodent mammalian species in addition to the rat or mouse. Studies may well continue over the major part of the lifetime of the rodent and for at least one year (generally two years) in tests with the dog or monkey. The common practice is to administer at least three dosage levels of the substance under test to each species in the expectation of finding both a no-adverse-effect level and a dose that induces some abnormal response. The number of animals routinely assigned to each experimental group of rats has increased from 5 or 6 in the early days to the present practice of using 25 of each sex, with a view toward increasing the statistical validity of the findings, especially at threshold dosages. The number of available biochemical indices of effect has increased from a few simple blood and urine determinations to a score or more that include levels of metabolites, electrolytes, and enzymes in body fluids and tissues. In many instances metabolic and functional tests are conducted. Effects on all aspects of reproductive physiology, with emphasis on the possibility of teratogenesis and on lactation, are investigated intensively. Lifetime studies are conducted to detect carcinogenic potential.

Histopathologic observations are now made on biopsy material as well as on material obtained postmortem and on many more tissues and organs than were examined in the early years. Electron microscopy, in addition to light microscopy, is being employed to detect

ultrastructural changes. Studies of the absorption, storage, excretion, and biotransformation of chemicals in the animal body are often an essential feature of toxicological assessments, and behavioral studies may be of use in such evaluations. In the future it will doubtless become customary to employ an even larger number of increasingly exacting tests for adverse reactions in animal species as a prerequisite for safety evaluation, at least of food chemicals that are proposed for extensive use.

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Evaluation of Conventional Methods

In 1959, the Food Protection Committee described the procedures then deemed most applicable to the evaluation of the safety of food chemicals.[1] After considering anticipated amounts and patterns of consumption of food chemicals, as well as their physical and chemical properties, the toxicologic aspects of the evaluation were described in terms of studies in rats and dogs of acute oral toxicity, subacute oral toxicity, and chronic (2-year) oral toxicity. The principal basis for the evaluation of safety became the determination of what was then termed the "no-effect" level in the 2-year study with rats and dogs. From this no-effect level, incorporating a substantial safety factor, an estimate could be made of a safe level of intake for man. In general, a no-effect level was regarded as one that produced no disturbance in growth, no clinical illness, no change in the mortality rate or pattern, no adverse biochemical or physiological effect, and no evidence, by gross and microscopic examination, of damage to body tissues or organs. Finally, this level of intake had no observed adverse effects on the reproduction of the rat.

On *a priori* grounds there is a calculated risk in basing a judgment with respect to the human species on the responses of any two non-human species. Nevertheless, the principles and procedures described in 1959 appear to have served well. Authenticated cases of human injury resulting from the presence of chemicals in foods as a consequence of approved usage in production, processing, packaging, or storage are extremely rare, indicating that the older methods have been effective; they should not be casually abandoned in favor of approaches that may be attractive only because they are new and different.

The classic 2-year chronic toxicity study took into consideration three things: that variation is to be expected among individuals within a single species; that variation is to be expected among species; and that a lifetime, or even several generations, of the test species might be required to demonstrate certain adverse effects.

Intraspecies variation was taken into account by insisting that enough animals be employed to validate the statistical significance of the results—in practice a minimum of 25 male and 25 female rats in each experimental group. Interspecies variation was taken into account by using the dog as comparison with the rat. The time required for the development of subtle effects and for the appearance of such slowly developing lesions as tumors was taken into account by extending the study over the approximate life span of the rat. Reproductive and teratogenic effects were sought through rat reproduction studies.

Tests of this kind are expensive and time-consuming, and they yield little information on just why or how the observed effects are produced. Methods developed since 1959 offer to supply some of these answers, but the crucial question is whether the newer methodology provides greater assurance of safety with smaller expenditure of time, scientific resources, and money, or whether they merely supplement the traditional methodology.

Since 1959 methods have been devised that will provide much more specific answers than those obtainable from chronic toxicity regardless of how carefully the latter are designed and conducted. The use of larger numbers of experimental animals and of more species will increase the precision of the observations that can be made but cannot supply information on mechanism of action. Among the principal areas in which the older methods fail to provide adequate answers are the following:

- In the absence of specific collateral studies, chronic studies do not provide knowledge of either the metabolic pathways followed by a compound or the physiological response induced by metabolic overloading. Adverse effects from relatively high dosage levels (i.e., meta-

bolic overloading) may be accorded (and in practice have been so accorded) undue importance in evaluating safety whereas, in fact, normal metabolism could safely cope with exposures at realistic use levels.

- The correlation between carcinogenic activity of chemicals in rodents and known chemical carcinogenesis in man is not sufficiently consistent to permit prediction of human hazard by direct translation from the effects in animals. Nonrodent species such as dogs, cats, and primates have life spans that make total lifetime exposure impractical, and there is therefore little, if any, information on carcinogenesis in these species to correlate with effects in man.
- In the complex environment of modern life, human beings are exposed to a multiplicity of chemicals. Although few instances of specific health hazard from the interaction of chemicals in the environment have shown up, there is always this possibility. Results from conventional methods rarely, if ever, provide insight into these possibilities.
- In general, conventional methods of estimating acute, subacute, and chronic toxicity do not bear on potential effects on reproductive function or on embryonic development. Modifications have been adopted to include these aspects, but further research is needed.

The above critique of conventional methods does not impugn their value, as established by experience. It does suggest that evaluation of the safety of food chemicals should be dynamic. As new methods are demonstrated to be more reliable than earlier ones, they should be adopted. Unless they meet this criterion, however, reliance must be placed on the conventional methods as refined by improved statistical control, by using animals of uniformly high quality, and by incorporation of advanced techniques.

It is essential that whatever tests are used they be performed on a sufficiently large number of animals of each species to ensure the desired level of statistical validity. At the present time no studies of short duration can be relied upon to predict the occurrence or nonoccurrence of such long-term effects as the induction of tumors. In spite of obvious disadvantages in terms of time, effort, and money, procedures for evaluating the safety of food chemicals recommended in 1959 cannot be considered obsolete.

It would be desirable indeed to have short, simple, and safe methods for evaluating the safety of food chemicals directly in the human species, but they are not now in sight. Studies on human subjects can be done under rigidly controlled conditions with reasonable safety, and results from such studies may well contribute to the overall evaluation

of safety. It is obvious, however, that in any use of human subjects the benefit in terms of knowledge to be gained should far outweigh the anticipated risk to the subjects, a matter that has recently been discussed in some detail.[2]

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Some Recent Approaches to Safety Evaluation

STUDIES ON THE METABOLIC FATE OF CHEMICALS *IN VIVO*

The effects of chemicals in various species can be considered from the point of view of the action of the chemical on the body or, conversely, of the action of the body on the chemical. The first is the principal objective of the search for the mechanism of action of a substance; the second is concerned mainly with the overall metabolism of the substance. For the purposes of this discussion, "metabolism" comprises everything that happens to the chemical during its stay in the body, including absorption from the intestinal tract, binding to proteins, transport through cell membranes, storage in various tissues, conversion to new substances (which may be either less toxic or more toxic), and excretion of the substance or its metabolites.

Metabolic studies, in general, long antedate their introduction into experimental toxicology. As early as 1830, Liebig proved that the substance present in the urine of horses that he called hippuric acid resulted from the combination of benzoic acid with a then unknown organic compound; shortly thereafter Keller showed that hippuric acid was produced in man from ingested benzoic acid.[1] By the beginning of the present century, the basic patterns of metabolic changes for many classes of organic compounds were relatively well understood, and a major topic during the early development of biochemistry was the metabolic fate of food substances.

One of the early applications of metabolic studies to food chemicals was that contained in the report of the Board of Scientific Advisors to the Secretary of Agriculture in 1911 on the safety of saccharin in man[2]—a detailed account of the nature and rate of excretion in urine. The development of sulfa drugs and of many other new pharmacologically active substances resulted, in the 1930's, in greatly increased use of metabolic studies in pharmacologic research. It was not until relatively recently, however, that this important approach received special attention in the study of food chemicals.[3]

The early lack of emphasis on metabolic studies is perhaps understandable in that their cost is high and the duration lengthy. Newer techniques, particularly the use of isotopes, various types of chromatography, and some of the newer developments in spectrometry, make it feasible to include metabolic studies in the ordinary course of development of toxicologic information about food chemicals. Along with the great increase in knowledge of chemical metabolism during recent years has come a realization that, when studied by modern techniques, the metabolic process is frequently more complex than had been previously thought and that it is not always possible to interpret the resulting data in terms of the safety of the compound.

Barnes and Denz,[4] in a critical review of the then current experimental methods for predicting cumulative effects of substances in man, said in effect that the toxicologist should determine not only whether a substance is toxic but, if so, why. Conversely, if a material is believed to be safe, evidence should be marshaled to support that view (e.g., evidence of metabolism and excretion). They advocated relatively short-term, high-dose studies to elicit any apparent toxic effects to be followed by intensive biochemical study with attention being given to the metabolism of the substance and to confirmation by studies on man whenever feasible. Only after this information is available, according to the authors, can other types of animal studies be properly designed. The report of the Food Protection Committee on the use of human subjects in safety evaluation[3] emphasizes much the same viewpoint and points out that one of the greatest needs at present is for better knowledge of the comparative metabolism of substances in various animal species and man.

Metabolic studies do not give direct evidence of the presence or absence of a toxic effect. It is instructive, however, to compare the toxicity and metabolism of foods, including their naturally occuring toxic

constituents, to those of chemicals that may be used in the production of foods. Evidence that the living organism uses similar metabolic pathways for any given chemical regardless of whether it is encountered in natural food or as a purely synthetic substance is overwhelming.

The ability of man to ingest with apparent impunity the nearly infinite variety of chemical substances found in his normal food is closely related to his ability to use them directly or to convert them to substances that are relatively easily excreted or stored without injury. It is apparent that man's adaptation to chemicals is related not only to his ability to handle them through the normal metabolic pathways associated with food components or endogenously produced substances, but also to the fact that substances never before encountered can be metabolized because of increased microsomal or other enzyme activity that may be induced following a short period of contact.[5] Although it is recognized that *qualitative* information about absorption, transport, storage, biotransformation, and excretion is important, we emphasize the fact that *rates* at which such processes occur often determine the differences among species and individuals in their responses to toxic materials. Experience with pharmaceutical agents provides many examples in which the rates of these processes determine the toxicity, side reactions, or effectiveness under given circumstances.[5] There is no reason to suppose that differences will not occur among animals used in toxicologic studies of chemicals, and they must be recognized in considering the safety of these chemicals for use in foods.

The following are areas in which information may be useful in assessing the safety of food chemicals:

Conversion to normal metabolic products. There are many instances in which lack of toxicity can be explained by the metabolic conversion of the substance to one or more products, the further metabolism of which is already known. One example is propylene glycol, a portion of which is converted to glycogen in the liver, whereas the remainder is excreted in the urine as an ester. The safety of many simple fatty acid esters is associated with their ready hydrolysis to the component alcohols and acids, which then enter the normal metabolic pathways. Many flavoring agents are esters of this category, a fact generally considered a strong indication of their safety.

Excretion, unchanged or in less toxic form. Rapid excretion cannot stand alone as a criterion of lack of toxicity, but there are so many examples from feeding studies of the close correlation between lack

of toxicity and rapid and complete excretion that the significance is obvious. A high margin of safety frequently appears to be related to rapid excretion of a material in the urine either unchanged or as some metabolite less toxic than the original substance. The conversion of benzoic acid to hippuric acid, which is then excreted in the urine, is an example. A number of phenolic substances are acetylated or conjugated and rapidly excreted in the urine. Nitrates appear to be extremely rapidly absorbed and excreted, as such, in the urine. Nitrate may be reduced to nitrite by the flora of the upper gut. This is usually of no consequence in the adult but, because of the greater susceptibility of infant hemoglobin, may be important in causing methemoglobinemia in infants. A number of dyes and larger molecules may be excreted via the biliary system rather than through the urine. Some solvents, for example, ethyl chloride and methylene chloride, are very rapidly and completely excreted in the expired air, apparently with no biochemical change. By contrast, substances such as benzene are converted to many diverse metabolites that are disposed of in a variety of ways. There is increasing evidence that the toxicity of carbon tetrachloride may be due to its conversion in the body to a free radical or other reactive metabolite.[6]

Formation of more toxic substances. Although most biotransformations of foreign organic chemicals result in less toxic compounds, the opposite occasionally occurs. The classical example is the conversion of fluoroacetate to fluorocitrate, which effectively interrupts the Krebs cycle and results in extraordinary toxicity. Other examples are the conversion of esters of thionophosphates to the corresponding phosphates, which are the actively toxic substances, and the conversion of nitrates to nitrites.

Metabolism and carcinogenesis. Recent reviews[7, 8] of carcinogenesis have emphasized that the action of most well-known carcinogens can be theoretically associated with the formation of "proximate" carcinogenic metabolites. Although information of this kind cannot yet be used directly in predicting carcinogenicity, research in this area should lead to improved predictability.

Evidence of nonabsorption from the intestinal tract. There are a number of substances, practically devoid of toxicity when taken orally, that are known to be not absorbed from the intestinal tract. Cellulose and a variety of artificially produced cellulose derivatives such as methylcellulose,[9] sodium carboxymethylcellulose,[10] and sodium cellulose sulfate,[11] are examples. The cellulose derivatives can be fed at very high levels in the diet without toxic effects other than those

related to reduction of nutrient intake or the induction of loose or bulky stools. Lack of absorption undoubtedly accounts for the nontoxicity of many insoluble substances (e.g., polyolefins, petroleum waxes, a wide variety of nonmetabolizable and nonabsorbable polymers, and barium sulfate and cadmium sulfide,[12] as well as of certain soluble salts (e.g., magnesium sulfate). Poor absorption is not always associated with lack of toxicity, however, because in some cases even a small absorption percentage of a chemical may be sufficient to produce toxic effects.

Storage in tissues. Evidence on the storage of a substance in the body may point to deposition in a particular organ, as, for example, the localization of mercury in the kidney or of lead in bones. The fact that storage occurs does not necessarily constitute a hazard; for example, the storage of DDT in fat has not apparently been associated with toxic reactions in human subjects who daily ingest small quantities of the chemical. Where storage in tissues has been thoroughly studied, as in the case of DDT or lead, there appears to be an equilibrium among uptake, storage, and excretion such that no obvious toxic effects occur at moderate levels of intake. Nevertheless, as a general rule, evidence that a material is tenaciously held in one or another organ of the body is cause for some concern and indicates the need for additional detailed investigation.

The transport of substances across body membranes. Sufficient information has now accumulated about the absorption of materials from the intestinal tract and about penetration through various cell membranes to allow useful theoretical analysis of the properties that may be expected to facilitate it. Reviews of this subject by Schanker[13] and Brodie[14] make it clear that lipid solubility is often an important factor in penetration of body membranes. If the material is ionizable, the portion un-ionized at the pH existing at the cell membrane surface penetrates most readily. Some small water-soluble molecules and naturally occurring metabolites can pass by diffusion through pores in cell membranes or by active transport. Protein binding is important in determining the rate and extent of renal excretion. These physicochemical factors, together with metabolic processes, may be critical in predicting what will happen with various chemical compounds.

The extensive background of work on the placental transfer of drugs may be helpful in considering possible effects of food chemicals. Villee[15] has pointed out that many lipid-soluble materials and a great variety of natural compounds readily cross the placenta. The rates of transfer of different lipid-soluble materials, however, vary considerably.

Knowledge of the ability of a chemical to cross the placenta and of its rate of transfer is obviously of help in evaluating possible effects on the fetus.

Problems of antagonism and synergism. Knowledge of the details of the metabolic alterations of substances may help explain some of the known cases of synergism and antagonism between chemical substances. An example is the ability of ethanol to lessen methanol toxicity. The preferential oxidation of ethanol inhibits oxidation of methanol in the liver and thus prevents the methanol from being converted to such more toxic substances as formic acid and permits it to be excreted unchanged. In the synergism between the two organophosphate insecticides, EPN and malathion, EPN inhibits the enzyme that destroys malathion, thus limiting the detoxification of the latter.

Simple additive effects are probably most likely when the substances have similar modes of action or when they have similar chemical structures and metabolic pathways. A metabolic antagonism may occur between a foreign substance and a substrate or co-enzyme of similar structure. In the classic case of Prontosil, the conversion to *p*-aminobenzene sulfonamide results in a competitive inhibition of the enzymes utilizing *p*-aminobenzoic acid, a growth factor for many microorganisms. Similarly, there are many examples in the drug literature of compounds that bear a sufficiently close resemblance to some naturally occurring hormone or chemical transmitter to be bound at an active site and that are thus able to block the action of the natural substance. Strong binding to an active site may result in antagonistic effects even when chemical structures are dissimilar.

In addition to the value of information on metabolism in predicting specific types of toxicity, there are other general uses. One of the principal uses for detailed information of this kind is related to epidemiologic studies, as in the exposure of persons to DDT. Samples of fat obtained at autopsy or from surgical procedures have given valuable clues as to the probable intakes of DDT by individuals and of the nature of the equilibrium reactions that are so important in evaluating the safety of this substance (see Reference 3).

Knowledge of metabolism increases the value of information that can be developed through occupational, medical, and industrial hygiene programs in industries manufacturing or using food chemicals. Intake by workers exposed under various conditions can best be estimated if the metabolism of the substance is known and if methods can be found for measuring the substance or its metabolites in blood, urine, or ex-

pired air. The foregoing applies also to accidental ingestion of substances. Knowledge of metabolism of the ingested substance would allow extraction of the maximum possible information from such incidents.

Pharmacogenetics is receiving emphasis in drug studies[16, 17, 18] and may be expected to yield insights into comparative toxicology. In the case of isoniazid, for example, the main pathway for detoxication is acetylation. Studies of blood levels of populations indicate that the distribution of values is bimodal. Although strictly genetic factors are not necessarily involved, they are suspected of being important. There have been similar findings in the case of the apnea induced by succinyl chlorine. The distribution of this condition apparently results from the fact that some members of the population hydrolyze the drug at a much slower rate than do others. Genotypes that result in deficiencies in glucose-6-phosphate dehydrogenase may be responsible for some drug-induced hemolytic anemias. Most of the examples developed thus far seem to involve a difference in rates rather than in types of metabolism, but this subject will be of increasing interest in the future.

The answers to the question of *why* substances are toxic will be approached increasingly by a combination of *in vitro* and *in vivo* methods of studying the interactions of chemicals with cells, tissues, or organs. However, evidence that a substance may be safe is not derived merely from a demonstration that at use levels it does not exert any of a variety of toxic effects seen at much higher levels. Confirmation of safety should be more concerned with elucidating the means by which the body is able to handle substances at characteristically low levels of intake over long periods without undue disturbance of, or stress on, body functions.

For ease of analysis, or sheer convenience, most metabolic studies have been carried out at levels of exposure far in excess of levels at which food chemicals are used. Such high levels often involve metabolic pathways that are distinct from those involved at low levels of exposure, or they result in the occurrence of the unmetabolized substance in body fluids or tissues because the capacity to detoxify or eliminate it has been exceeded. It can reasonably be expected that the population distribution of the metabolic processes, rates, and values associated with overloading will be entirely different from that associated with the more normal metabolic pathways at lower levels of intake.

The relevance of the presence or absence of toxic effects as seen in feeding studies to the prediction of safety at use levels can be determined with greatest certainty if the nature of metabolism is known at

both the toxic level and the use level. A toxic effect seen only at a level of exposure at which aberrant metabolism is present is not as helpful in estimating a safe use level as would be one appearing at levels at which normal metabolic patterns prevail. Accordingly, the toxicologist should give prime attention to the nature of the metabolic processes at levels of exposure near those to be anticipated in use and should attempt to evaluate those factors that bear on the ability of populations to tolerate substances at these levels. It is in this respect that study of the qualitative and quantitative aspects of metabolism will be most useful in the future.

BIOCHEMICAL AND *IN VITRO* STUDIES

Significant advances have been made in recent years in knowledge of the intracellular location of metabolic pathways in mammalian cells. The nature, properties, and intracellular location of enzyme systems that catalyze the biotransformation of substances foreign to the body have received a great deal of study. Evaluation of the safety of chemical agents can become increasingly more precise by the application of these advances in knowledge to the study of the biological effects and fates of food chemicals. The scope and nature of studies along these lines will necessarily differ from one chemical agent to another, a fact that makes it impossible to designate in advance a series of measurements to be done on a given compound. Direction for the type of investigations on the mechanistic aspects of a substance may come from the observations made during toxicity tests and studies on the pharmacological actions in the intact animal. An important objective of the application of recent advances in knowledge and methodology is to increase understanding of the mechanism of the toxic action, the role of biotransformation in determining the duration of action and persistence of the chemical agent, and the possible influence of the substance being examined on the actions or fates of other chemicals given simultaneously or at a later time.

A considerable amount of exploratory work, including the development of suitable methodology, may be necessary before an *in vitro* technique can be applied to the evaluation of the toxicity of food chemicals. It is thus apparent that the initial study of a chemical should be done in a species such as the rat, whose metabolic processes have been much studied. Treatment with relatively high single doses or repeated high doses usually yields animals well suited to examination for defects in cellular metabolism. Correlations of such defects with

morphological alterations observed by conventional histologic techniques, histochemical methods, and electron microscopy can then be attempted.

Electron microscopy is particularly important in that it can demonstrate changes that are not otherwise visible. The great magnification and resolution of the electron microscope allow the observation not only of structures that are just distinguishable with the light microscope, such as mitochondria, but also of those that are completely unseen with it, such as the cristae of various intracellular organelles and the ribosomes attached to the endoplasmic reticulum. Specific examples of contributions of electron microscopy to understanding of physiologic effects are to be found in References 19–23.

When significant biochemical or morphologic changes in experimental animals are coincident with high doses of a chemical, attempts should be made to ascertain whether the same effects occur at doses approximating levels that man would be exposed to under proposed conditions of use. Consideration should be given also to the possibility of devising direct or indirect methods for determining whether a particular change observed in experimental animals occurs in man. However, the practical limitations of any such study in man must be recognized in the final assessment of safety.

Results of some of the newer *in vitro* approaches to studies of the mode of action of chemical agents cannot be interpreted clearly in terms of similar changes in the intact animal. For example, the use of tissue and cell cultures is frequently suggested as an approach to measurement of cell injury. However, the pronounced influence that one organ system—such as the liver, the kidney, or an endocrine gland—or the intact animal *in toto*, may exert on the duration, intensity, and type of action of a chemical agent *in vivo* makes it necessary to consider findings in isolated cell systems as no more than suggestive of experiments that might profitably be conducted.

Research on the mechanistic aspects of the toxicology of food chemicals should be clearly differentiated from the application of a series of routine biochemical tests to the urine and blood of animals and man. The latter procedures are primarily diagnostic aids in revealing injury to certain organ systems, but they may also point toward mechanistic studies in a particular organ. Negative findings in these routine tests indicate only that the chemical under investigation does not affect the particular processes that were examined. Intensive research on the mode of toxic action of chemical agents on a molecular, *in vitro* basis may not elucidate the exact mode of action prior to

introduction of the agent into actual use. However, such studies could in many instances provide a better understanding of the compatibility of the agent with biological systems than can be obtained from conventional methods.

In safety evaluation, consideration should be given the hepatic microsomal enzyme systems that catalyze metabolism of many relatively nonpolar chemicals. The role of these enzymes in metabolizing a food chemical can be studied, as can the ability of the chemical to induce synthesis of microsomal enzymes. Both of these types of investigations should first be conducted in experimental animals. *In vitro* studies can then be done by adding the food chemical to a microsomal enzyme system and identifying metabolites by chemical or physical methods. In this connection, a certain amount of predictability concerning the types of changes that will occur is made possible by knowledge that has been developed during the past few years. *In vitro* studies will indicate the types of metabolites that might be expected to appear in the blood, bile, and urine. The alternative, detecting and identifying metabolites in urine and blood without prior *in vitro* studies, may provide the most practical procedure for measuring the rate and extent of metabolism of a food chemical in man.

The ability of a food chemical to induce synthesis of microsomal enzymes can be determined by multiple dosing of animals for several days followed by assays for microsomal enzyme activity in the liver. The effect can then be studied in other species, including man, by determining, both before and after administration of the chemical for several days, the blood levels and rate of excretion of the same or another chemical agent known to be metabolized by these enzymes. The no-effect level for enzyme induction can be measured by performance of enzyme assays on the livers of animals fed various levels of the food chemical. This type of information will aid in ensuring that the permitted levels are incapable of altering the toxicity or duration of action of chemicals to which animals and man may be exposed.

DESIGN OF STUDIES TO DEVELOP TESTS APPLICABLE TO MAN AND PROPERLY CHOSEN ANIMAL SPECIES

During the past decade, there have been several developments bearing upon safety evaluation. One of these is the use of the electron microscope to provide detailed information about changes in structure of various organelles within cells as a result of administering chemicals to an experimental animal or human subject. In some cases, it has been

possible to show in experimental animals that a single, large oral dose of a chemical produces within one hour a recognizable change in the morphology of some type of parenchymal cells. This kind of evidence leads rapidly to the target organs or tissues affected by the chemical under study and allows the investigator so to plan his subsequent investigation as to furnish detailed knowledge of the actions of the chemical on the target sites.

The initial change is not necessarily one that results in damage to the organ or tissue affected; whether it results in a deleterious structural change will await subsequent study. If the initial change is purely functional, it may be reflected in the increased production of the drug-metabolizing enzymes within the endoplasmic reticulum of the cell or the microsomes of homogenates. Mitochondrial enzymes also may be altered.

In developing tests with animal species designed to yield results applicable to man, it is important to find a species that metabolizes and excretes a given chemical as nearly as possible in the same manner as does man. In many cases, however, an ideal experimental animal cannot readily be identified, and studies must be made in less desirable species. Of the many species of laboratory animals now available, the most useful are probably the mouse, rat, hamster, guinea pig, rabbit, dog, pig, and monkey. No precise rule for choosing animal species for study can be stated.

To select an animal species suitable for detailed study, a number of test species may be given repeated sublethal doses of the test chemical during a period of several days, their urine and feces being analyzed for metabolites. It may then be feasible to employ a few conservative tests in man, using single oral doses, to allow comparative examination of urine and feces. If the metabolic pattern in man is similar to that of one of the species tested, then that species may well be chosen for intensive studies of its reactions, both immediate and delayed, to repeated administration of the test compound, as evidenced by biochemical or ultrastructural changes. Prediction that the toxic effects observed in the test species might occur in man may be made on the basis of blood or tissue concentrations after exposure of man and the test species to various doses of the chemical, as suggested by Brodie.[24]

After completion of the toxicity studies, usually within 3 to 6 months after the animal species is selected for intensive study, feeding tests in man may be undertaken. In these tests, the chemical should be used as in its projected role in the diet. The subjects should be given thorough physical examinations. Base-line values should be established in appropriate tissue specimens and body fluids for those factors that underwent significant alterations in the test animals. The trials in man should last for at least 3 to 6 weeks, and can be expected to establish whether the previous choice of an animal species as being reasonably similar to man was correct and to indicate the likelihood of significant effects in man from the new chemical if used in food.

PHYSIOLOGIC STUDIES

Reproduction Conventional studies on fertility and reproduction are carried out in rodents. However, since the female monkey or chimpanzee, like the human, has a menstrual rather than an estrus cycle, they may be of particular value.

It is important to study the effects of proposed food chemicals on the maturation of both ovum and sperm. Effects on reproductive efficiency can be exercised also through changes, for example, in libido, receptiveness, vaginal or prostatic pH, and implantation. These factors can be studied experimentally in laboratory animals, and some of them can be studied in man during the trials discussed earlier.

Females becoming pregnant during the study should be examined near or at term to assess implantation, resorption, and other aspects of embryonic development. Females not exposed to the chemical and made pregnant at known times should be dosed during various crucial stages of pregnancy, e.g., for 1-month periods, starting 2, 6, 10, 14, or 18 weeks after fertilization, in the monkey, or for 1-week periods, starting 1, 8, 16, or 25 days after copulation, in the rabbit.

Placental Transfer If pregnancy proceeds normally despite administration of the test chemical, appropriate studies to define the placental transmission of the material to the fetus may be undertaken. Such studies might involve nothing more than estimation of the rate of accumulation of the chemical or its metabolites in fetuses, or they might involve more sophisticated studies of simultaneous levels of the chemical and its metabolites in maternal and fetal bloods or of the rate of appearance of radioactively labeled chemical in fetal blood following its injection into the maternal circulation.

Lactation Studies of the effects of the chemical on the amount and quality of milk produced may be carried out. Transmission of the chemical through milk to the newborn animal is an important factor for study.

The possibility of increased susceptibility of the lactating animal to a chemical should be considered. These toxicity determinations should be made in previously unexposed females at intervals during lactation. When the chemical is administered as a proportion of a diet fed *ad lib.*, the response to increased intake because of increased food consumption should not be confused with enhanced toxicity.

Teratogenesis Teratogenic chemicals act on the developing embryo in such a way as to produce congenital malformations that are ordinarily not heritable. Though only a few substances have been demonstrated to be teratogenic for man, a large number have been shown under experimental conditions to produce fetal abnormalities in laboratory animals.[25, 26] Variations in experimentally induced teratogenic response in rats, rabbits, and mice, and the lack of correlation with human experiences raise serious questions concerning species and strain specificity. Recent knowledge regarding the mechanisms of congenital malformations was presented in 1968 at the Second International Workshop in Teratology.[27]

Assuming that an ingested chemical has teratogenic potential and gains access to the embryo, the main factors determining whether teratogenesis will, in fact, occur are (a) dosage, (b) the species of animal, and (c) the stage of development of the embryo at the time of contact with the chemical.

Experience in experimental teratology has shown that a substance that is teratogenic in one species may not be so in another and that not all strains of a given species respond alike, nor do all individuals of the same litter. Hence the presence or absence of a teratogenic effect in one or more animal species exposed to a chemical is of limited value in predicting whether the chemical will be teratogenic for man. As in all toxicologic work, however, the consistent finding of such an effect in several species strengthens the suspicion (but cannot prove) that man, too, would be susceptible. Conversely, consistent absence of the effect in laboratory animals gives some assurance, but does not prove, that man would not be affected.

The sensitivity of the embryo to teratogenic change is restricted to the period of cellular differentiation and organogenesis. Different tissues or embryonic sites are susceptible to changes at different stages of development of the embryo, and the times of the peak sensitivity vary within the narrow limits of only a few days during gestation. When organogenesis is completed, true teratogenesis can no longer occur, though the fetus is still subject to toxic effects that may influence its later growth or survival.

Most experimental teratology has employed the mouse, the rat, and the rabbit. Since the chemical under investigation must be given to the pregnant female before and during the period when fetal cell differentiation and organogenesis are in progress, the time of conception must be precisely known. Interpretation of the results should be conditioned by knowledge of the time course of absorption, distribution, metabolism, and excretion of the chemical. The relation between the concentrations of the chemical in the maternal blood and in the embryo are critical in evaluating its teratogenicity.

The chick embryo is also used for teratology studies. In this case, the embryo is exposed to controlled concentrations of the chemical by injecting the chemical into the incubating egg. Although this test may reveal a teratogenic potential of a food chemical, the results obtained may have but limited relevance to the evaluation of teratogenic hazard for man, not only because of species variation but also because of the likely differences in the concentration of the acutely administered and chronically ingested chemical at the critical stage and sites of embryogenesis.

The initial observations of possible teratogenic effect are generally made during appropriate multigeneration reproduction studies in mammals. These studies should be designed with adequate numbers of females so that some can be delivered by cesarean section to permit observation of implantations and resorptions and examination of intact fetuses. If any teratogenic effect or other aberration is indicated, specifically designed tests in which the timing of conception and dosage are controlled should be carried out. The most critical test involves the administration of the chemical to the pregnant animal at the period of maximum sensitivity during embryogenesis. The rate of *in vivo* biotransformation of many chemicals may either increase or decrease during the course of repeated intake. For this reason, the teratogenic potential of a food chemical administered continuously during a reproduction study may differ from that in a teratology test in which the chemical is given only at the estimated time of maximum embryonic sensitivity.

Little is yet known about toxicity to the developing embryo, the transport of chemicals to it, the biochemical mechanisms controlling embryogenesis, and the factors that influence the sensitivity of the processes of cellular differentiation to disruption by foreign chemicals.

Considerable progress in this area is necessary before the teratogenic hazard of food chemicals for man can be reliably evaluated.

Mutagenesis Mutagenesis differs from the other categories of embryotoxicity in that the defect, if not lethal, has the potential for reappearing in generations succeeding that in which it is first seen. Chemicals may alter not only fertilization and intrauterine development of fertilized ova, but also the hereditary material itself (mutagenesis). Although they may be manifested in similar ways, genetic aberrations are to be distinguished from other types of impairment of reproductive function, including blastotoxicity, resorption, abortion, teratogenesis, and impaired regulatory or functional mechanisms (e.g., abnormal enzyme or protein constitution, hormone imbalance, and carcinogenesis).

Although radiation has been the most commonly recognized mutagen, a number of chemicals have been found to be mutagenic *in vitro* to cultured cells or *in vivo* to experimental species. No instance of chemical mutagenesis in man seems to be definitely known. Nevertheless, the similarity of the hereditary materials in different animal species and the ability of nonlethal mutations to affect generations following the one in which the change is first observed raise the question of whether mutations may not be induced in man by chemicals added to food.

Tests for mutagenic action in mammals are in an early state of development. An *in vitro* procedure that can be used as an initial screening test consists of exposing human leukocytes in a culture fluid to graded concentrations of the chemical. After a period of incubation, mitosis is stopped at metaphase by the addition of colchicine. The cells are then stained and examined for altered chromosomes. Whether to accept such changes as evidence of mutation is subject to sharp differences of opinion.

The intact animal possesses mechanisms that afford protection against toxic substances, for example, limited absorbability or detoxication within the gastrointestinal tract or after absorption. Because cells *in vitro* lack these protective mechanisms, the substances found to induce chromosomal change by an *in vitro* test should be examined for possible mutagenic effects in the intact anima'. The host-mediated assay, which has attracted considerable attention, is an attempt to bridge this gap. A microbiological indicator (*Neurospora candida* spores or bacterial cells) is administered intraperitoneally to a host mammal while the host is exposed by a different route to the chemical under consideration. The microorganism is withdrawn after a suitable period and compared with *in vitro* grown cultures for evidence of induction of mutations.

If reproduction studies, carried out through three or more offspring generations, are negative, they tend to rule out mutagenic as well as other embryotoxic effects. If the indexes of reproductive performance are abnormal in the multigeneration reproduction test, definitive tests should be carried out to identify the cause.

With respect to mutagenesis, a widely used first test is the dominant lethal test in which treated and untreated males are mated with untreated females. The pregnant females are then sacrificed and the uteri examined for the number of implantation sites, dead fetuses, and living fetuses. Present evidence indicates that, at least in mice, rats, and guinea pigs, there is no correlation between the number of corpora lutea in the ovaries and the number of implantation sites.

A variation of the dominant lethal test that has gained considerable support involves treating maturing male mice (8 to 9 weeks of age) with the chemical under test and mating them with normal females during four successive weeks of spermatogenesis. Ten days after mating, the females are autopsied and the number of viable fetuses counted.[28,29] The evaluation of potential mutagenic effects during spermatogenesis is believed to have special value for detecting antifertility effects that are not associated with apparent structural damage to testicular cells.[29]

A more critical application of the multigeneration test involves feeding the initial F_0 generation throughout its life cycle and observing it for a dominant effect, and following the second litter of each generation to at least F_6. If a recessive effect appears, progeny are backcrossed with parent generations to confirm it.[30]

It is obvious that improved methods are needed for assessing mutagenesis, which can produce such conditions as alcaptonuria, phenylketonuria, galactosemia, sickle cell anemia, mongolism, and dizygotic twinning in man. As a corollary, research should be performed not only on the possible mutagenic effects of chemicals to be added to foods but also on ways whereby their mutagenic activities in man can be predicted accurately.

Environmental Stresses That various environmental stresses may have additive effects on the toxicities of chemical agents has been recognized for many years, but it has not been practical to study the possible relationships extensively. However, since most chemical agents are studied in several laboratories that may differ from each other in the method

of housing, in the strains of animals used, and even in the composition of the diets used, similarity of results obtained in different laboratories indicates that at least subtle to moderate differences in the environment do not affect the toxicity of a chemical agent. Special consideration of stress conditions is indicated, however, when it is known that the food chemical would be used under conditions unusual with respect to diet, environment, or disease.

Age Differences A comparison of the susceptibility of animals of different ages should be a regular part of toxicity evaluation. Particular attention should be given to measuring differences between infants and adults, because incomplete development of enzymes in the very young of some species is known to affect their responses to chemical agents. The time relationships have been less clearly established for other species, including man, but age differences in susceptibility have been observed in enough different species to indicate the advisability of carefully considering this factor.

Psychopharmacology Many chemicals affect the central nervous system. The effects range from local (interference with the perception of some particular sensory input) to general intoxication (hallucination, aimless and perseverative activity, coma, etc.).

Routine psychopharmacologic testing of food chemicals is not suggested, but this is an area of active research, and where such study is indicated new food chemicals may be examined not only for direct psychotropic action but also for ability to enhance the effects of other compounds.[31]

Methods of studying psychotropic actions in experimental animals are extremely varied. They range from the use of chronically implanted electrodes to record from, or to stimulate, particular loci within the central nervous system, to sophisticated tests of ability to perform alternatively different sequences of responses (see References 32–44).

A thorough study of the behavioral effects of a chemical requires the use of more than one animal species in a statistically adequate design. Because genetic and cultural backgrounds of animal populations are important determinants of responses to chemicals, the rat has distinct advantages over most other species of laboratory animals for psychopharmacologic research. Other species, however, have been used.[45, 46]

Whatever species of laboratory animal is used, the research should be planned to relate change in behavior quantitatively to the independent variable (dose of chemical or time between administration of the chemical and observation). To do this, the independent variable must be presented in at least two (preferably three) values and the behavioral (dependent) effects must be measured quantitatively. The psychopharmacologist must keep in mind also that behavior can be altered by nonspecific, peripheral actions.

When human studies of psychopharmacologic effects have been undertaken, the following tests, among others, have been used:

Visual acuity
Estimation of elapsed time
Dynamometer
Romberg's sign
Simple reaction time
Manual dexterity
Sequential addition or subtraction
Repetition of a series of numbers

Other tests, such as canceling certain numbers within arrays of random numbers, circling a specified type of word on pages of print, or drawing lines to connect serially numbered points, may replace the last two items in the battery of tests or may be added to it.

Both animal and human tests must be adjusted to the properties of the particular compound under study. Certain tests may have to be altered or replaced by others after preliminary studies have been made. It is obvious that the methods employed in psychopharmacological study must be as varied as the behavioral responses to administered chemicals.

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Evaluating Carcinogenic Potential

In many, if not most cases, the carcinogenic potential of a chemical is assessed using the same animals employed for assessing other toxic hazards. Carcinogenicity deserves special treatment, however, because of the frequently long induction periods required before tumors can be detected and, in the United States at least, because of the special emphasis given to it by the Food, Drug, and Cosmetic Law.

The Food Protection Committee[1] and the FAO/WHO Joint Expert Committee on Food Additives[2] reviewed the matter of evaluation of carcinogenic potential of food chemicals in 1959 and 1961, respectively. The following presents contemporary views on this matter.

GENERAL CONSIDERATIONS

Routes of Application Although chemical carcinogens can be administered in a variety of ways, the three routes that have been most used are subcutaneous, oral, and topical. Parenteral routes, such as intraperitoneal and intravenous injection, and the direct injection of compounds into, or their implantation within, specific tissues have also been employed. Insofar as possible, tests to assess the carcinogenic hazard of a chemical for man should utilize conditions similar to those that might obtain during human exposure. Compounds that might

nter via the alimentary tract should be tested by oral administration, nd those that would come into contact with the skin or lungs should e tested by topical application or inhalation. Except in special situa-ons, the latter two routes are of little relevance to human exposure o food chemicals.

Carcinogens that are administered in the ultimate carcinogenic form, r that can be metabolized to such a form at the injection site, are sually administered by single or repeated subcutaneous injection. lowever, the results of subcutaneous injection in the commonly used odents must be treated with caution. In many cases production of rcomata in these tests seems to be related to physiochemical prop-ties of the substance injected and to special conditions of the experi-ent. For example, "smooth surface carcinogenesis" occurs in rats and me other species in connective tissue cells near intact implants of in pieces of sufficient area of almost every substance tried (cello-hane, nylon, Teflon, ivory, silver, platinum, etc.), and the weak car-nogenic activity of repeated injections of cholesterol in the rat has een ascribed to the surfaces of the cholesterol crystals so deposited. general, the production of sarcomata at injection site appears not be related to the chemical composition of the implants and is con-dered not to be evidence of carcinogenicity by the oral route.[3]

The oral route is the most likely mode of entry into man of food emicals (including pesticide and herbicide residues), many drugs, d water pollutants. The carcinogenicity of aromatic amines has been ost readily demonstrated by chronic oral administration. The total nounts of compounds administered often have had to be large, prob-ly because of limited conversion to the ultimate carcinogenic metab-ite and limited delivery to the critical targets.

se Response In general, the incidence of tumors increases and the ent period decreases as the dose of a carcinogenic chemical is in-eased. Even when the carcinogenic potential of a chemical at a given se level has been demonstrated, with smaller dose levels the latent riod may be too long to permit gross tumors to develop within the span of the animal, and a "no carcinogenic level" may thus be wn for that species. Conversely, high doses may kill the animal fore tumors become apparent.

mbined Effects of Two or More Carcinogens Doses of the same or different carcinogens administered either simultaneously or sequen-lly may produce additive, synergistic, or inhibitory effects. Inhibi-tory effects can result from altered metabolic patterns that reduce the effective levels of one or both carcinogens (inhibition of carcinogenesis by amines through administration of polycyclic hydrocarbons is an example). Competition for reactive sites may be important in other situations. The best documented synergistic responses are found in mouse skin tumorigenesis. Thus, initiating agents (e.g., urethan) and promoting agents (e.g., croton oil) may induce very few tumors when applied alone, but application of an initiator and then a promoter results in high tumor yields.

Rapid Tests for Carcinogenic Activity At present only the production of tumors in the intact animal is recognized as a valid test of carcinogenic activity. Since these tests require many months or years, more rapid assays to predict the possible carcinogenic hazard of chemicals have been sought. To date no test with good predictive value for chemicals of different types has been developed, although in some cases correlations have been achieved within restricted classes of compounds. Chemical transformation of cells cultured *in vitro* has been studied in this context, since at least some clones from such transformed cells grow as tumors *in vivo*. Assays for destruction of sebaceous glands of mouse skin and various mutagenicity and toxicity tests in plants, in *Drosophila*, and in microorganisms have also been proposed as short-term tests for carcinogenicity.

Two major questions are pertinent to all these tests: Is the same reactive form the key intermediate in the assay under consideration and in the carcinogenic action of the chemical? and Are the capabilities of the test cells and the various tissues of man to make the reactive metabolite similar? These questions are difficult enough to answer with known chemical carcinogens, but impossible with compounds whose carcinogenic potential is unknown.

Test Procedures in the Detection of Carcinogenic Activity of Food Chemicals In view of differences in susceptibility, it is generally recommended that at least two species be used for tests on each chemical. Furthermore, special conditions of immaturity, pregnancy, sex, other drugs, etc. that might be involved in human exposures should be simulated in at least some of the tests. The number of people with tumors of any type at any one time in the United States approximates 3 per 1,000 population. Most tumors occur in older individuals who have been at risk for extended periods. Experimental groups of animals large enough to detect tumor incidences of this order of magnitude

are clearly not feasible. Hence, lifetime experimental dosage levels far exceeding the probable human exposure levels are employed to compensate partially for this logistical problem. In general, it is recommended that the doses administered be 10 to 1,000 times those that could be achieved in human exposure, if this is pharmacologically and practically possible. However, it must be recognized that compounds administered in massive doses may be metabolized by pathways different from those that characterize low levels of intake.

In view of the importance often attached to low incidence of tumors in the treated animals, it is essential that the number of animals assigned to each group be as large as practicable and that most of the nontumor-bearing animals survive for most of their expected life span. Several dosage levels of each test compound should be used so that information on the relationship between tumor incidence and dose is available, especially when spontaneous tumors occur in the animal strain employed.

It is critical that all animals be autopsied at death or upon the termination of the experiment and that they be subjected to careful gross and microscopic examination. Of equal importance is the maintenance at the same time of large groups of control animals that receive treatment identical to that of the experimental groups except for administration of the compound under test; this includes feeding the same diet and applying the vehicle used for administering the test compound. The control animals must be autopsied comparably to those of the experimental group.

Oral Administration Compounds can be administered by incorporation into the diet, dosage being controlled by weighing food intake, or by gastric intubation, which permits more accurate dosage and is preferable for labile compounds. Stable water-soluble compounds can also be administered in the drinking water. Diets containing test compounds should be made up frequently and stored so as to avoid rancidity or destruction of the test compound. When compounds are administered in the drinking water, the water should be changed daily. In both cases records of consumption should be kept so that total intakes can be calculated.

The highest dosage employed should be one that will produce a minimal to moderate amount of short-term toxicity but that will permit most of the animals to survive for at least 1 year. At least one or two lower doses that will not cause significant reduction in the life span should also be administered. Unless the survival of the animals is threatened, administration of the compounds should continue for most of the life span.

The rat, mouse, and hamster have been widely used in studies of the carcinogenicity of orally administered compounds; because of this and their relatively short life spans, they are probably the animals of choice.

Pathological Examinations Regardless of the species, compound, or route of administration, pathological examination of all animals is of paramount importance. For this purpose it is essential that moribund animals or those with large tumors be sacrificied and that dead animals be removed for necropsy as soon as possible. All animals should receive complete necropsies that include careful gross examination of the skin and its underlying structures; the mammary tissue; the abdominal, thoracic, pelvic, and oral glands; and endocrine and nervous systems. All tumors or possible tumors should be fixed and examined by a competent pathologist familiar with the species under study. Control animals must receive comparable examinations to ascertain the significance of the tumors observed in the experimental groups.

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Interpretation and Prediction of Safety

FACTORS AFFECTING PREDICTION

The principles and procedures for safety evaluations recommended in 1959 have, insofar as we know, served well in protecting the public from health hazards that might otherwise have resulted from use of chemicals in food production, processing, storage, and packaging. It has been widely felt, however, that these conventional procedures are overly cumbersome, time-consuming, wasteful of manpower and resources, and expensive, and that they do not provide the basic information needed to advance the efficiency and dependability of safety evaluation.

Although toxicologic knowledge and understanding increased greatly in the 10 years since 1959, the requirements for reaching a judgment of safety became, at the same time, more rigorous, with the result that no savings in time, manpower, and money were realized. Although there has been a trend toward streamlining of the conventional acute, subacute, and chronic toxicity tests, there has appeared no reliable alternative to the test for carcinogenic potential, which is the chief time-consumer. In addition, reproduction studies are now almost routinely required, and it is increasingly urged that observations on teratogenicity and mutagenicity be made. One can anticipate that clinical tests will be expanded and that histological examination will become more extensive and detailed than is now generally the case.

The increased complexity of the methodology of safety evaluations has come about because of our continuing ignorance of the basic mechanisms of the actions of chemicals in the body, and is enhanced by our ignorance of the significance of the results of many of the new tests for the body economy. However, recently developed knowledge of the basic biochemical actions of chemicals and new insights into these actions promise an improvement in both the economy and reliability of safety evaluation. As more knowledge is gained about the comparative metabolism of chemicals in various species and about the basic biochemical, structural, and functional changes induced by chemicals, there should be a concomitant increase in the usefulness of data derived from study of one species for guiding judgments of safety in another.

PREDICTION OF SAFETY FOR MAN FROM OBSERVATIONS ON ANIMALS

Physiological and biochemical similarities among mammals permit one to measure many indexes in laboratory species that can then be predictively transposed to man. The opportunity to exaggerate both the dosage and duration of administration or exposure in animal studies has special value as contrasted to human tests and makes such studies mandatory whether or not tests or observations in man are undertaken. Despite the interspecies similarities, however, there are many metabolic and organic differences that cast a shadow of uncertainty on species-to-species extrapolations. Although the uncertainty inherent in such extrapolation tends to diminish as the number of animal species showing identical responses increases, it never vanishes completely. In the last analysis, experience and scientific judgment must be relied upon in applying experimental data to conditions of use of food chemicals.

Present knowledge does not ensure absolute protection against injury from food chemicals—or indeed from any environmental hazard—for all individuals in the population. The population includes hypersensitive or allergic individuals, persons with bizarre food habits, and persons in different age groups and physiologic and disease states. Most safety evaluation studies are conducted in healthy animals receiving normal diets, and the results are probably most applicable to prediction of events in healthy human beings on good diets. There is much need for research to extend the validity of conclusions to the more extreme or abnormal human situations.

The determination of a dosage level, ideally the maximum dosage

level, that is without discernible adverse effect in animal species is of decisive importance in safety evaluations since it is this dose (expressed in relation to body weight or surface) that is translated, after applying a suitable "safety factor," into a tolerable (or maximum acceptable) intake for man. The no-adverse-effect dose can be estimated to a degree sufficient to provide virtual certainty of safety but can never be absolute. The reasons for the residual uncertainty are numerous and include the following:

- The size of the test groups is small relative to the population to which the data are to be applied.
- A certain degree of risk (probability factor) is inherent in any findings depending on a finite number of animals.
- No-adverse-effect levels vary with the species, strain, age, sex, physiologic state, etc., of the test animals, hence the failure to observe an adverse effect under one set of defined experimental conditions does not preclude the possibility of an effect under other conditions.
- No matter how many types of response may be observed in toxicologic studies, it is always conceivable that one or more tests not employed in a particular study, or not even devised, might reveal an effect not previously observed.
- Although no adverse effect means no toxic effect, it is difficult sometimes to decide whether an aberration, i.e., a difference from a "normal" or control response, is indeed an adverse effect. For example, an elevated level of a normal constituent in blood or tissue, an increase in organ weight, or a so-called nonspecific histomorphologic alteration, unaccompanied by dysfunction, may or may not be indicative of disease or injury.

SELECTION OF SPECIES

After acute and short-term toxicity measurements in several animal species and preliminary studies of the fate of a food chemical in these species and in man have been completed, species for the special and long-term evaluation procedures might be selected, based on three considerations: the most sensitive species, the most convenient species, and the species with a metabolic pattern most similar to that of man.

Sensitivity Use of results from tests in the most sensitive species for predicting how man will be affected has little scientific merit unless it is already known that man resembles that species in his reaction to the chemical. In setting tolerances and in regulatory control, however, it seems sensible to use the no-effect level in the most sensitive of the species appropriately tested, even though the practice is conservative, involving uncertainties in extrapolating information to man and the assumption that man may be equally as sensitive as, or more sensitive than, the experimental animal.

Convenience Selection of species on the basis of convenience, i.e., because of availability, familiarity, ease of handling, length of life span, size, cost, etc., has been widely resorted to. In the first place, it is highly practical and from such a selection one can choose the most sensitive species for the long-term tests and for the computation of tolerance. Furthermore, after studies of the fate of the chemical have been accomplished in the various species chosen, the one most closely simulating man in this regard can then be subjected to long-term and other special studies.

Similarity in Metabolism Choice of an animal species with a metabolic pattern similar to that of man has substantial scientific merit for reasons noted above, but it is likely to present difficulties.[1] It presupposes that sufficient animal studies have already been done to justify investigating the fate of the chemical in man. Hopefully, one of the species selected for the first stages of the evaluation will prove to simulate man in this regard. If none does, then further exploration would have to be done. If a suitable species is thus identified for use in special and long-term studies, before such studies are undertaken acute and short-term toxicity tests would have to be done. Should the species thus identified turn out to be a relatively infrequently used animal or a large animal, such problems as procurement and housing will in many cases outweigh the scientific advantages. Enthusiasm for the concept of pairing an animal species with man on the basis of metabolic similarities must be tempered by the realization that both may exhibit marked variability in the metabolism of chemicals, depending on age, sex, genetic factors, and physiologic state.

PREDICTION OF SAFETY FOR MAN FROM OBSERVATIONS ON MAN

The ideal species in which to study safety of food chemicals for man is man himself. The nature, usefulness, and limitations of using man in such studies have been succinctly discussed.[2, 3]

The ultimate assessment of the safety of a food chemical derives from years of widespread consumption by man under given conditions of use. Even here, however, the absence of known adverse effects does not, by itself, constitute adequate assurance of safety. The possibility always exists of adverse effects that, because of their subtle or slowly developing nature, are not recognized as being caused by the chemical in question.

Observations on occupationally exposed workers and on accidentally overexposed individuals can provide reasonable support for a judgment of safety, especially if the exposures are excessive and still cause no detectable adverse effects. Under these circumstances, however, the conditions do not simulate the proposed use of the food chemical in respect either to the route of intake or the schedule and duration of exposure.

Carefully controlled epidemiological studies in which the observed subjects consume the food chemical under conditions of its proposed use are notably difficult to carry out. They are subject to the pitfalls inherent in the complex interplay of multiple factors—intake, age, sex, race, dietary habits, and variable physiological, social, and environmental circumstances. To conduct a meaningful survey requires large numbers of subjects and of physiological measurements. If the distribution of products containing a new food chemical could be initially restricted to a limited geographic area, epidemiologic studies could be done much more easily than is true under the more customary practice of nearly universal distribution. Practically, however, restricted distribution would, in most instances, not be long enough to provide reliable information on the possible development of infrequently occurring or slowly developing ill effects.

Controlled experimental studies in man, though desirable, have limited predictive value for a number of reasons:

- The feasible duration of the experiment is but a relatively short segment of man's total life span. Thus, observations relating to carcinogenesis are impossible in the light of present knowledge and methodology.
- The number of subjects is usually not adequate to reveal serious reactions that occur but rarely.
- In extended studies, the low levels of a food chemical administered, as dictated by ethical considerations, do not provide data from which a range of safety can be computed. No degree of risk can be measured. Neither the toxicity nor the degree of safety of food chemicals

can readily be measured quantitatively in man in a controlled experiment that meets accepted ethical standards.

- To control accurately the intake of the chemical, it is frequently given by capsule or in some other manner not precisely that proposed for use in foods.
- Lapses in the cooperation of the subject weaken the dependability of the data.
- Tests are usually carried out on a specific category of subjects, such as healthy adult males, and do not make allowances for the variable susceptibilities that are related to sex, pregnancy, age, race, and environmental factors.
- The variety of experimental tests that can legitimately be used is limited in comparison to those that can be employed in experimental animals.
- Because the subjects are informed of the experiment and of any potential risk, psychological reactions may distort the results, unless the experiment is carefully designed.

It is clear that appropriate and well-executed animal studies are more likely to provide a substantial background of biological data from which to judge safety than are studies in man alone.

At the same time, recent developments in the study of the fate of chemicals in man promise to provide data than can be of prime predictive value. A comparison of the patterns of disposition of a food chemical in man and in several animal species might well bring to light an ideal experimental animal in which to carry out the necessary extensive toxicologic studies from which data may be obtained that can be extrapolated back to man with a comparatively high degree of reliability.

Man himself may exhibit individual quantitative or qualitative variations in disposing of a chemical, variations resulting from differences in age, sex, physiologic state, inheritance, or environment. In general, however, if an animal with a relatively short life span and a pattern of metabolism and excretion similar to that of man can be identified, the results of a well designed life-span toxicity test in that species can be accorded greater value for the prediction of safety in man than can the results of a test of simular duration and best possible design in man.

In spite of the limited predictive value of studies in man, the maximum level of assurance of safety would be achieved by the combination of the following: (1) extensive studies of toxicology, mechanism of action, and metabolic fate in appropriately selected animal species;

(2) suitably controlled testing procedures in man; (3) epidemiological studies; and (4) several years of monitored exposure of man. But even the conscientious exploitation of this program of study will not completely dispel the uncertainty about harmful effects that are difficult to attribute to specific causes, because they develop slowly, are of a subtle nature, or occur infrequently.

IN VITRO MODELS

Chemicals have been applied *in vitro* directly to living cells, to tissues or organs, or to various simple organisms, in seeking to elucidate their toxicity and mechanisms of action. Chicken eggs and embryos have been used in studies of teratology and toxicology, animal and human leukocytes; *Drosophila* in testing for mutagenicity; tissue slices and homogenates for enzyme studies; and cell and tissue cultures for study of growth and structural changes. These techniques have frequently been of value in certain assay procedures and in the study of the basic mechanisms of interaction between chemicals and cells or cell constituents. Results serve to guide the investigators' observations of the whole animal, particularly when the *in vitro* system is prepared from the same species of animal as that used for *in vivo* study. In evaluating the safety of a new food chemical, data obtained from an *in vitro* system cannot be directly extrapolated to the *in vivo* situation in either qualitative or quantitative terms with any degree of certainty. Hence, the results of *in vitro* toxicity tests cannot be said to have direct predictive value, because of the following factors:

- The effects of the gastrointestinal environment upon the chemical are absent.
- Factors that would limit absorption of the chemical from the intestine in the intact animal are missing.
- The *in vitro* system may not metabolize (activate or inactivate) the chemical.
- Excretory mechanisms are lacking.
- Responses to stress and homeostatic and adaptive mechanisms are missing.
- Distribution and transport factors are missing, and the *in vitro* model may not simulate the critical target organ.
- Duration of exposure to the chemical in the *in vitro* system cannot accurately simulate chronic exposure of the whole animal.

Notwithstanding these limitations, the potential of *in vitro* investigations as a guide to more definitive tests justifies continued search for ways in which they can be of value.

EVALUATION OF CARCINOGENIC HAZARDS OF CHEMICALS FOR MAN

On both ethical and practical grounds, the possible carcinogenic effects of chemicals to which man might be exposed must be determined in experimental animals, generally in short-lived species. The extrapolation of the results of these determinations to man rests on two basic findings:

1. Despite a great variety of studies, no significant aspect of the natural occurrence, induction, and properties of cancer has been shown to differ fundamentally between man and experimental animals.
2. Considerable assurance of the general applicability of the tests can be derived from the fact that the chemicals generally known to be carcinogenic in man also induce neoplasia in experimental animals.

Many clear differences in the activities of chemical carcinogens in various species exist, however, and the unpredictable nature of species differences is the principal limitation to the extrapolation of results from studies with experimental animals to man.

In order to minimize the species (i.e., metabolic) differences between man and experimental animals in their responses to a chemical compound, tests for carcinogenicity must be conducted with dosages at the highest practical levels—i.e., at the highest level that does not overload the normal metabolic pathway or greatly decrease life span—and in more than one test species. As knowledge of the mechanisms of chemical carcinogenesis increases, information derived from metabolic experiments with tissue preparations from human beings and experimental animals may make it possible to select the best species for the lifetime test of a given chemical. At present, however, the uncertainties make it necessary to evaluate both positive and negative results in these tests with considerable caution.

Evidence that a substance is carcinogenic in one or more experimental species raises the suspicion that it would be carcinogenic in man. Although there are probably noncarcinogenic dosage levels for an experimental species in which a given compound is carcinogenic, there is

as yet no generally accepted way of quantitatively extrapolating dose-response data in predicting a noncarcinogenic level for man. This fact is the principal basis for urging that such compounds be permitted for use in foods only if an explicit judgment has been made that demonstrable benefit greatly exceeds the risk.

There is unavoidable uncertainty about the safety of a substance not found to be carcinogenic in experimental species, the degree of which must be judged for each compound on the basis of the stringency of the tests used and the expected exposure of man to the compound in question. This uncertainty must be balanced against the benefits to be gained from the proposed uses of the substance.

Unless there is prior knowledge of the carcinogenic potential of a substance that is proposed for addition to the environment, particularly if large numbers of human beings of all ages might be exposed to it for appreciable portions of their life spans, tests adequate to demonstrate safety in this regard are necessary. Safety of each substance must be evaluated on results of such tests and a knowledge of the projected uses.

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Appendix: Guidelines for Estimating Toxicologically Insignificant Levels of Chemicals in Food*

In 1958, the Food Protection Committee issued a statement[1] that pointed out that for every chemical there is some finite level, sometimes called the "safe level,"[2] at or below which it can be present in food without prejudicing safety. The safe level of a substance is usually established through extensive toxicological study and expert evaluation. It is generally set by applying some factor, called the "safety factor," to the highest dietary intake that is found not to injure experimental animals exposed under close observation for extended periods. Thus, the safe level is frequently expressed as 1/100 of the experimentally determined "no-adverse-effect level." This ratio, 1:100, provides a conservative estimate of the safety factor needed to afford adequate protection, even to persons whose dietary patterns or individual susceptibilities are unusual. This procedure has been so widely adopted by national and international bodies concerned with food safety that the term "safe level" as applied to chemicals in food is generally understood in the sense set forth here.

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[1] Insignificant levels of chemical additives in foods. 1958. Food Drug Cosmetic Law J. 13:477–479.

[2] The "safe level" in the sense here discussed refers to the maximum acceptable daily intake in the total diet. It is not to be equated with "tolerances," which are legally established limits to the concentration of chemicals permitted in agricultural products or foods compatible with good agricultural or manufacturing practice. Tolerances are established with due consideration of safe levels.

Uses of a chemical that in total contribute a level to the diet close to the "safe level" should be regulated to prevent risk to the consumer from avoidable or adventitious excesses. The 1958 statement referred to above recognized that when any particular use results in a dietary level well below the safe level, the possibility of hazard from use or misuse becomes so remote that regulatory activity to protect the public from the chemical in question is superfluous. Such low levels are sufficiently presumptive of safety that they may reasonably be termed "toxicologically insignificant." No attempt was made in 1958 to define "toxicologically insignificant" in quantitative terms. In this statement we now propose guidelines for quantitatively defining levels of chemicals in food that can be administratively considered toxicologically insignificant.

It is axiomatic that no substance should be allowed to enter food unless there is convincing evidence that the amount used will be safe beyond reasonable doubt. This principle is embodied in food regulations (21 CFR 121.1 i) that define safety as the reasonable certainty that no harm will result from the intended use of the food additive.

In the period since the Committee's 1958 statement, a considerable body of new or additional toxicological information has accumulated about many chemicals added to foods, the metabolic processes by which the body deals with them, and their acceptable levels of intake. For administrative and regulatory purposes, the safe level has generally been derived in the manner outlined above as a conservative fraction of the "no-adverse-effect level" found in animal testing. However, if a level in the feeding test that permits survival of at least some of the animals for the duration of the test is found to produce cancer, it may not, according to the Food, Drug, and Cosmetic Law, be used in any concentration in or on food. An observation of any such serious condition makes it prudent to consider the nature of the dose–response relationship and the physiologic, metabolic, or pathologic processes involved to ensure against the possibility that the same effect might occur in man.

The vast majority of the chemical entities that we consume are present naturally and unavoidably in foods. Only a small proportion of these substances have so far been identified, and an even smaller proportion have been investigated by conventional toxicological procedures. The levels at which most of them are usually consumed are assumed to be toxicologically insignificant because (1) they have been consumed by man for generations without apparent harm; (2) they are present in foods at extremely low concentrations; and (3) insofar as is known, they are not related chemically to substances of known high toxicity. Thus, based on these criteria, there is a body of empirical knowledge deemed sufficient on which to base a judgment of the safety of virtually all these substances at the levels found in food. The latter two criteria are equally valid when applied to evaluation of safety for use of synthetic substances.

THE NEED FOR PRIORITIES

Because of the thousands of natural and synthetic substances present in food at low and intermediate levels, it becomes necessary to establish a reasonable system of priorities for the further study of those substances not yet fully evaluated. To study every chemical to the same extent as those that must be used at close to their safe levels would represent an unjustifiable expenditure of effort not contributing significantly to protection of public health. It is neither practicable nor necessary to undertake experimental toxicological studies of every chemical to which man is exposed; to do so would be to assign equal importance to problems of unequal risk. This would deny the value of experience in assessing probable risk. All environmental exposures must be subjected to scientific evaluation, but not all exposures require experimental toxicological study.

To provide optimum assurance of public safety within the limitations of capabilities available, toxicological facilities for evaluating safety must be concentrated on environmental situations in which there is a reasonable expectation that exposure to chemicals may cause real hazards. The definition of a "toxicologically insignificant" level simply as one well below an established safe level is too indefinite and too limiting to be of practical value, since it requires in every case the establishment of a safe level by experiment. To insist that nothing can be assumed to be safe without direct experimental toxicological evidence implies that safety must be proved experimentally before the proof of safety can be considered unnecessary. This denies the value of establishing criteria for insignificance. Thus, there is urgent need to arrive at more specific guidelines for estimating dietary levels that can be considered toxicologically insignificant.

This report endorses and extends the application of the term "negligible" as employed by a previous committee of the National Academy of Sciences–National Research Council,[3] which recommended that uses contributing only a small fraction of the safe dietary level, as previously defined, be ignored administratively. But it is concerned in addition with dietary levels of chemicals for which safe levels have not been determined in the laboratory.

THE APPLICATION OF EXPERIENCE

Our search for increasingly toxic pesticides or antipersonnel agents, for drugs active at low dosages, and for naturally occurring toxins has had the expected result of revealing substances that demonstrate toxicity at lower levels than any substances previously known. Thus, if distinction among probable hazards is ignored, the

[3] Report of the Pesticide Residues Committee, June 1965. A Report of the National Academy of Sciences–National Research Council to the Food and Drug Administration and the U.S. Department of Agriculture.

concept of an insignificant level would drift downward to essentially zero. For the practical reasons outlined above, however, we cannot afford to ignore the distinctions, outlined in the following, that increasing experience now permits us to make.

Tabulation and examination of compounds in commercial use that may have deleterious effects at low levels reveal that they fall into four general categories:

1. Certain impurities of contaminants of natural origin;
2. Certain essential nutrients and hormones;
3. Certain heavy metals and their compounds; and
4. Certain organic compounds employed for their biological activity.

In the first category are such substances as aflatoxin, botulinus toxin, and tetrodotoxin, which are toxic at concentrations as low as 0.001 ppm. In categories 2, 3, and 4 are a large number of pesticides, pharmaceuticals, and antipersonnel agents that may have biological activity at levels as low as 0.1 ppm. Aside from these classes of compounds, no commercial compound has been demonstrated to produce toxic reactions below a dietary concentration of 40 ppm.[4]

Obviously, the level that can be considered toxicologically insignificant for one category may not, for that reason, be so considered for another category. The criteria for insignificance will vary for different classes of compounds and may change with further research on compounds within classes. Chemicals that exert significant biological effects and that are useful or unavoidable in food or any other part of the environment will continue to be subjected to laboratory investigations to establish safe levels, and the experience gained thereby will constantly provide the basis for confirming or modifying the earlier conclusions.

Synthetic organic chemicals that are not manufactured specifically for their biological activity must be sharply distinguished from naturally occurring toxins or trace contaminants. During development and production of a new commercial chemical, some form of toxicological testing is a commonplace precaution. Additionally, a degree of biological knowledge is gained through human exposures in development and production of a new chemical that is more extensive than the knowledge we have of most trace contaminants. It is virtually certain that unpredicted effects of extremely toxic compounds would be revealed through exposure in the work environment, and such compounds would then be placed in category 4 of the above tabulation.*

The toxicology of substances intentionally used for their biological activity is always investigated experimentally to establish limits of their safe usage.

ESTIMATION OF TOXICOLOGICALLY INSIGNIFICANT LEVELS

It is generally true that exposure by nonoral routes may not be a reliable basis for predicting a maximum safe dietary level. Nevertheless, unavoidable industrial

[4] J. P. Frawley. 1967. Scientific evidence and common sense as a basis for food-packaging regulations. Food Cosmet. Toxicol. 5:293-308.

*That is, if they are commercially developed.

experience provides useful guidance that can be applied to evaluating safety in the diet, including judgment as to toxicological insignificance for various classes of substances, based on available information concerning their safety or that of related compounds.

Except for the categories of compounds cited under 2, 3, and 4, no single organic chemical that has advanced from the laboratory, through development, into general commercial use has been demonstrated to be toxic to experimental animals at a dietary level of 40 ppm or less. Compounds that possess greater toxicity have either been developed specifically for use as economic poisons (as defined by the law), as drugs, or as chemical warfare agents, etc., or were found to possess such biological activity during development and were diverted to these uses.

A. Chemicals in Commercial Production

If a chemical has been in commercial production for a substantial period, e.g., 5 years or more, without evidence of toxicological hazard incident to its production or use, if it is not a heavy metal or a compound of a heavy metal, and if it is not intended for use because of its biological activity, it is consistent with sound toxicological judgment to conclude that a level of 0.1 ppm of the chemical in the diet of man is toxicologically insignificant.

B. Pesticide Degradation Products

Most degradation products of pesticides are less toxic than the parent material. A few exceptions have been experienced, but even in these cases the toxicity of the degradation products is only slightly greater than that of the parent compound. If the safe level of the pesticide is 1 ppm or above, it appears to be a safe working guideline that dietary levels of degradation products below 0.1 ppm are insignificant and undeserving of laboratory investigation.

C. Organic Chemicals Lacking Toxicological Data, but Meeting Special Structural Restrictions

For many substances that are functionally effective in food at dietary concentrations above 0.1 ppm, but still much below any reasonable judgment as to their maximum safe level, as previously defined, there is need to arrive at estimates of toxicologically insignificant levels. For these substances, it is justifiable to employ accumulated scientific experience and to recognize their structural analogy to other chemicals whose metabolism or toxicity is known. Reasoning by analogy may be used to arrive at conclusions of toxicological insignificance. If a substance meets *all* the following criteria, it may be presumed to be toxicologically insignificant at a level of 1.0 ppm or less in the human diet:

1. The substance in question is of known structure and purity;

2. It is structurally simple[5];

3. The structure suggests that the substance will be readily handled through known metabolic pathways; and

4. It is a member of a closely related group of substances that, without known exception, are, or can be presumed to be, low in toxicity.[6]

D. Organic Chemicals with Minimal Toxicological Data and Less Closely Related Structures

In cases nearly, but not precisely, meeting the above criteria, a level of insignificance may be established if

1. There are available adequate scientific studies that establish safe levels of similar magnitude for at least two analogous substances.

2. The acute or subacute toxicity of the new substance and the analogous substances is of the same nature and degree.

A sound estimate of the safe level of the new substances that meets the two foregoing conditions is the lowest safe level of all the analogous substances that have been studied. If the safe levels for all the structurally analogous substances are essentially identical, 1/10 of the estimated safe level may be taken as a toxicologically insignificant level. In the event of appreciable differences among the safe levels of the analogous substances, the insigificant level should be taken as 1/20 of the estimated safe level.

In conclusion, it is important to protection of public health that responsible and reasonable priorities be established to guide research efforts in toxicology. The principle of toxicological insignificance is a valid concept for separating potential health hazards from predictably safe applications. Guidelines on toxicological insignificance are needed to eliminate wasteful diversion of scientific resources in university, industry, and government laboratories. The levels judged to be insignificant by this Committee are conservatively derived from accumulated toxicological experience. They are intended to guide and stimulate—not replace—informed professional and administrative judgment. Like all other judgments of toxicologic hazards, they should not be considered infallible against future evidence; and, as with any experimentally derived toxicologic information applied to evaluation of safety for man, continuous awareness of possible unexpected effects must be maintained. However, in light of today's knowledge, application of these guidelines will protect public health and simultaneously allow greater attention to be devoted to projects of greater potential significance to health.

[5] Examples that are intended to be illustrative, not limiting, are
(a) Straight-chain or simply branched aliphatic alcohols, acids, and esters.
(b) Linear polymers of ethylene or ethylene oxide.
(c) Cellulose ethers.
(d) Mononuclear aromatic compounds containing only carbon, hydrogen, and oxygen, and equipped with one or more functional groups that include hydroxyl, aldehyde, and keto.

[6] "Closely related" shall be understood to mean
(a) Near members of a homologous series.
(b) Geometric or positional isomers that would not be expected to present serious differences in chemical reactivity or steric effects.
(c) Substances of identical basic structure or differing only by (a) or (b) above, and possessing additional functional groups readily accommodated by known metabolic mechanisms.
(d) Compounds readily metabolized into substances meeting the other criteria here listed.